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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<p>(54) Title: METHOD OF TREATMENT FOR LUNG DISEASES USING ANTISENSE OLIGONUCLEOTIDES</p> <p>(57) Abstract</p> <p>A method of treating airway disease in a subject in need of such treatment is disclosed. The method comprises topically administering to the subject an antisense oligonucleotide in an amount effective to treat the airway disease, where the antisense oligonucleotide is essentially free of adenosine. Pharmaceutical formulations are also disclosed.</p>			

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## METHOD OF TREATMENT FOR LUNG DISEASES USING ANTISENSE OLIGONUCLEOTIDES

This invention was made with Government support under grant RO1CA47217-06 from the National Cancer Institute. The Government has certain rights to this invention.

### 5 Field of the Invention

This application concerns a method of administering antisense oligonucleotides essentially free of adenosine as a treatment for lung diseases.

### Background of the Invention

10 Antisense oligonucleotides have received considerable theoretical consideration as potentially useful pharmacologic agents in human disease. R. Wagner, *Nature* 372, 333-335 (1994). However, practical applications of these molecules in actual models of human disease have been elusive. One important consideration in the pharmacologic application of these molecules is route of administration. Most experiments utilizing antisense oligonucleotides *in vivo* have involved direct application to limited regions of the brain (see C. 15 Wahlestedt, *Trends in Pharmacological Sciences* 15, 42-46 (1994); J. Lai et al., *Neuroreport* 5, 1049-1052 (1994); K. Standifer et al., *Neuron* 12, 805-810 (1994); A. Akabayashi et al., *Brain Research* 21, 55-61 (1994)), or to spinal fluid (see e.g. L. Tseng et al., *European J. 20 Pharmacol.* 258, R1-3 (1994); R. Raffa et al., *European J. Pharmacol.* 258, R5-7 (1994); F. Gillardon et al., *European J. Neurosci.* 6, 880-884 (1994)). Such 25 applications have limited clinical utility due to their invasive nature.

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The systemic administration of antisense oligonucleotides also poses significant problems with respect to pharmacologic application, not the least of which is the difficulty in targeting disease-involved 5 tissues. In contrast, the lung is an excellent potential target for antisense oligonucleotide application since it may be approached noninvasively and in a tissue-specific manner. Additionally, the lung represents an exceptional target for antisense ODN therapeutics as compared to other 10 *in vivo* target organs or tissues, possibly because the lung is lined with surfactant which consists primarily of cationic lipids, well known to enhance cellular uptake of ODNs in other systems. However, the technology involved in delivering antisense agents to the lung remains 15 relatively undeveloped, and potential problems related to the application of antisense agents to the lung remain unexplored.

Adenosine, a purine which contributes to intermediary metabolism and participates in the 20 regulation of physiological activity, is a recognized neuromodulator. This nucleoside is involved in many local regulatory mechanisms, in particular at synapses in the CNS and at neuroeffector junctions in the periphery. In the CNS adenosine is known to inhibit the release of 25 a variety of neurotransmitters (noradrenaline, serotonin, GABA, acetylcholine, dopamine, glutamate, etc.), to inhibit neurotransmission, depress neuronal firing, induce spinal analgesia, and to possess anxiolytic properties (E.S. Ben-Sorek et al., *Archives of Internal 30 Medicine* 153, 2701-2702 (1993)). In the heart, adenosine is known to slow atrioventricular (AV) conduction, suppress pacemaker activity, possess antiarrhythmic effects, modulate autonomic control, and to trigger the synthesis and release of prostaglandins. M.K. Church et 35 al., *J. Allergy & Clinical Immunology* 92, 190-194 (1993). It also possesses potent vasodilatory effects and modulates vascular tone. S.T. Holgate et al., *Annals*

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of the New York Academy of Sciences 629, 227-236 (1991).

As a therapeutic agent, adenosine has achieved considerable recent success as an antiarrythmic agent in 5 the treatment of supraventricular tachycardia. See C.G. DeGroff and M.J. Silka, *Journal of Pediatrics* 125, 822-823 (1994); I. Drake et al., *Human and Exp. Toxicol.* 13, 263-265 (1994). However, many adverse effects of adenosine treatment have been reported in the literature. 10 See, e.g., A. Aggarwal, et al., *Anesthesiology* 79, 1132-1135 (1993); K.K. Burkhart, *American J. Emergency Med.* 11, 249-250 (1993); S.K. Srinivasan and P.J. Iversen, *J. Clin. Lab. Analysis* 9, 129-137 (1995); C.A. Stein et al., *Pharmacology & Therapeutics* 52, 365-384 (1991); B.B. 15 Fredholm et al., *Pharmacological Reviews* 46, 143-156 (1994); H. Saito, et al., *Blood* 66, 1233-1240 (1985). In particular, asthmatic individuals show an extreme sensitivity to adenosine and adenosine monophosphate. See, J.H. Butterfield et al., *Leukemia Res.* 12, 345-355 20 (1988); *CLONETICS: Normal Human Cell Systems Manual* (1995); R.W. Wagner, *Nature* 372, 333-335 (1994). Serious, near-fatal induction of bronchospasm has occurred in asthmatic individuals administered adenosine for supraventricular tachycardia. See, S. Tabor, in: 25 *Current Protocols in Molecular Biology*, Vol. 1, Section 3.10.2 (John Wiley & Sons, 1987); J.H. Weiss, *Id.*, at Section 6.2.2.

Similarly, asthmatic rabbits produced using the dust mite allergic rabbit model of human asthma also were 30 shown to respond to aerosolized adenosine with marked bronchoconstriction, while non asthmatic rabbits showed no response. S. Ali et al., *Agents Actions* 37, 165-176 (1992). Recent work using this model system has suggested that adenosine-induced bronchoconstriction and bronchial 35 hyperresponsiveness in asthma are mediated primarily through the stimulation of adenosine receptors. S. Ali et

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al., *J. Pharmacol. Exp. Ther.* 268, 1328-1334 (1994); S. Ali et al., *Am. J. Physiol* 266, L271-277 (1994).

Accordingly, adenosine is contraindicated in the lungs of asthmatics (who represent 10% of the adult 5 and 15% of the pediatric population in the United States). Since antisense ODNs are typically composed of all four base pairs, adenine, guanine, cytosine and thymidine, their breakdown products will produce free deoxyadenosine monophosphate in these hyperresponsive 10 airways. Deoxyadenosine monophosphate differs from adenosine monophosphate only by the loss of an oxygen atom on the 3' carbon of the sugar moiety.

#### Summary of the Invention

15 A first aspect of the present invention is a method of treating airway disease in a subject in need of such treatment. The method comprises administering an antisense oligonucleotide essentially free of adenosine to the lungs of the subject in an amount effective to treat the airway disease.

20 A second aspect of the present invention is a pharmaceutical composition, comprising, together in a pharmaceutically acceptable carrier, an antisense oligonucleotide essentially free of adenosine in an amount effective to treat an airway disease.

25 A third aspect of the present invention is the use of an antisense oligonucleotide essentially free of adenosine as given above for the preparation of a medicament for treating airway disease in a subject in need of such treatment.

#### Brief Description of the Drawings

Figures 1-4 demonstrate that antisense oligonucleotides can be utilized as effective agents in the treatment or prevention of airway diseases.

30 Figure 1 illustrates the effects of A<sub>1</sub> adenosine receptor antisense oligonucleotides and mismatch control

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antisense oligonucleotides on the dynamic compliance of the bronchial airway in a rabbit model. Figure 2 illustrates the specificity of A<sub>1</sub> adenosine receptor antisense oligonucleotides as indicated by the A<sub>1</sub> and A<sub>2</sub> 5 adenosine receptor number present in A<sub>1</sub> adenosine receptor antisense oligonucleotide-treated airway tissue.

Figure 3 is a graphical representation illustrating that aerosolized deoxyadenosine monophosphate is a potent bronchoconstrictor in asthmatic 10 pathways of allergic rabbits. Further, the figure shows that the effect of deoxyadenosine monophosphate is equipotent to that observed for adenosine monophosphate.

Figure 4 is a graphical representation illustrating that bronchoconstrictor effects occur with 15 aerosolized phosphorothioate oligodeoxynucleotides containing adenosine, but not with oligodeoxynucleotides that are free of adenosine.

#### Detailed Description of the Invention

Nucleotide sequences are presented herein by 20 single strand only, in the 5' to 3' direction, from left to right. Nucleotides and amino acids are represented herein in the manner recommended by the IUPAC-IUB Biochemical Nomenclature Commission, or (for amino acids) by three letter code, in accordance with 37 CFR §1.822 25 and established usage. See, e.g., PatentIn User Manual, 99-102 (Nov. 1990) (U.S. Patent and Trademark Office, Office of the Assistant Commissioner for Patents, Washington, D.C. 20231); U.S. Patent No. 4,871,670 to Hudson et al. at Col. 3 · lines 20-43 (applicants 30 specifically intend that the disclosure of this and all other patent references cited herein be incorporated herein by reference).

The method of the present invention may be used to treat airway disease in a subject for any reason, with 35 the intention that adenosine content of antisense compounds be eliminated or reduced so as to prevent its

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liberation upon antisense degredation. Such liberation may cause serious, even life-threatening, bronchoconstriction in patients with hyperreactive airways. Examples of airway diseases that may be treated 5 by the method of the present invention include cystic fibrosis, asthma, chronic obstructive pulmonary disease, bronchitis, and other airway diseases characterized by an inflammatory response.

Antisense oligonucleotides to the A<sub>1</sub> and A<sub>3</sub> receptors are shown to be effective in the downregulation 10 of A<sub>1</sub> or A<sub>3</sub> in the cell. One novel feature of this treatment, as compared to traditional treatments for adenosine-induced bronchoconstriction, is that administration is direct to the lungs. Additionally, a 15 receptor protein itself is reduced in amount, rather than merely interacting with a drug, and toxicity is reduced. Other proteins that may be targeted with antisense agents for the treatment of lung conditions include, but are not limited to: human A2a adenosine receptor, human A2b 20 adenosine receptor, human IgE receptor  $\beta$ , human Fc-epsilon receptor CD23 antigen, human histidine decarboxylase, human beta tryptase, human tryptase-I, human prostaglandin D synthase, human cyclooxygenase-2, human eosinophil cationic protein, human eosinophil 25 derived neurotoxin, human eosinophil peroxidase, human intercellular adhesion molecule-1 (ICAM-1), human vascular cell adhesion molecule 1 (VCAM-1), human endothelial leukocyte adhesion molecule (ELAM-1), human P selectin, human endothelial monocyte activating factor, 30 human IL-3, human IL-4, human IL-5, human IL-6, human IL-8, human monocyte-derived neutrophil chemotactic factor, human neutrophil elastase, human neutrophil oxidase factor, human cathepsin G, human defensin 1, human defensin 3, human macrophage inflammatory protein-1- 35 alpha, human muscarinic acetylcholine receptor HM1, human muscarinic acetylcholine receptor HM3, human fibronectin, human GM-CSF, human tumor necrosis factor  $\alpha$ , human

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leukotriene C4 synthase, human major basic protein, and human endothelin 1. In these latter targets, and in target genes in general, it is particularly imperative to eliminate or reduce the adenosine content of the 5 corresponding antisense oligonucleotide to prevent their breakdown products from liberating adenosine.

As used herein, the term "treat" or "treating" a lung disease refers to a treatment which decreases the likelihood that the subject administered such treatment 10 will manifest symptoms of the lung disease. The term "downregulate" refers to inducing a decrease in production, secretion or availability (and thus a decrease in concentration) of the targeted intracellular protein.

15 The present invention is concerned primarily with the treatment of human subjects but may also be employed for the treatment of other mammalian subjects, such as dogs and cats, for veterinary purposes. Targeted proteins are preferably mammalian and more preferably of 20 the same species as the subject being treated.

In general, "antisense" refers to the use of small, synthetic oligonucleotides, resembling single-stranded DNA, to inhibit gene expression by inhibiting the function of the target messenger RNA (mRNA). 25 Milligan, J.F. et al., *J. Med. Chem.* 36(14), 1923-1937 (1993). In the present invention, inhibition of gene expression of the A<sub>1</sub> or A<sub>2</sub> adenosine receptor is desired. Gene expression is inhibited through hybridization to coding (sense) sequences in a specific messenger RNA 30 (mRNA) target by hydrogen bonding according to Watson-Crick base pairing rules. The mechanism of antisense inhibition is that the exogenously applied oligonucleotides decrease the mRNA or protein levels of the target gene or cause changes in the growth 35 characteristics or shapes of the cells. *Id.* See also Helene, C. and Toulme, J., *Biochim. Biophys. Acta* 1049, 99-125 (1990); Cohen, J.S., Ed., *Oligodeoxynucleotides as*

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*Antisense Inhibitors of Gene Expression; CRC Press:Boca Raton, FL (1987).*

As used herein, "antisense oligonucleotide" is defined as a short sequence of synthetic nucleotides that 5 (1) hybridizes to any coding sequence in an mRNA which codes for the targeted protein, according to hybridization conditions described below, and (2) upon hybridization causes a decrease in gene expression of the targeted protein.

10 The mRNA sequence of the A<sub>1</sub> or A<sub>3</sub> adenosine receptor is derived from the DNA base sequence of the gene expressing either the A<sub>1</sub> or A<sub>3</sub> adenosine receptor. The sequence of the genomic human A<sub>1</sub> adenosine receptor is known and is disclosed in U.S. Patent No. 5,320,963 to G. 15 Stiles et al. The A<sub>3</sub> adenosine receptor has been cloned, sequenced and expressed in rat (see F. Zhou et al., *Proc. Nat'l Acad. Sci. USA* 89:7432 (1992)) and human (see M.A. Jacobson et al., U.K. Patent Application No. 9304582.1 (1993)). Thus, antisense oligonucleotides that 20 downregulate the production of the A<sub>1</sub> or A<sub>3</sub> adenosine receptor may be produced in accordance with standard techniques.

One aspect of this invention is an antisense 25 oligonucleotide having a sequence capable of binding specifically with any sequence of an mRNA molecule which encodes an airway disease-associated protein so as to prevent translation of the mRNA molecule.

Chemical analogs of oligonucleotides (e.g., 30 oligonucleotides in which the phosphodiester bonds have been modified, e.g., to the methylphosphonate, the phosphotriester, the phosphorothioate, the phosphorodithioate, or the phosphoramidate, so as to render the oligonucleotide more stable *in vivo*) are also 35 an aspect of the present invention. The naturally occurring phosphodiester linkages in oligonucleotides are susceptible to degradation by endogenously occurring cellular nucleases, while many analogous linkages are

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highly resistant to nuclease degradation. See Milligan et al., and Cohen, J.S., *supra*. Protection from degradation can be achieved by use of a "3'-end cap" strategy by which nuclease-resistant linkages are 5 substituted for phosphodiester linkages at the 3' end of the oligonucleotide. See Tidd, D.M. and Warenius, H.M., *Br. J. Cancer* 60, 343-350 (1989); Shaw, J.P. et al., *Nucleic Acids Res.* 19, 747-750 (1991). Phosphoramidates, phosphorothioates, and methylphosphonate linkages all 10 function adequately in this manner. More extensive modification of the phosphodiester backbone has been shown to impart stability and may allow for enhanced affinity and increased cellular permeation of oligonucleotides. See Milligan, et al., *supra*. Many 15 different chemical strategies have been employed to replace the entire phosphodiester backbone with novel linkages. *Id.* Backbone analogues include phosphorothioate, phosphorodithioate, methylphosphonate, phosphoramidate, boranophosphate, phosphotriester, 20 formacetal, 3'-thioformacetal, 5'-thioformacetal, 5'-thioether, carbonate, 5'-N-carbamate, sulfate, sulfonate, sulfamate, sulfonamide, sulfone, sulfite, sulfoxide, sulfide, hydroxylamine, methylene(methylimino) (MMI) or 25 methyleneoxy(methylimino) (MOMI) linkages. Phosphorothioate and methylphosphonate-modified oligonucleotides are particularly preferred due to their availability through automated oligonucleotide synthesis. *Id.* Where appropriate, the antisense oligonucleotides 30 may be administered in the form of their pharmaceutically acceptable salts.

Antisense oligonucleotides may be of any suitable length (e.g., from about 10 to 60 nucleotides in length), depending on the particular target being bound and the mode of delivery thereof. Preferably the 35 antisense oligonucleotide is directed to an mRNA region containing a junction between intron and exon. Where the antisense oligonucleotide is directed to an intron/exon

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junction, it may either entirely overlie the junction or may be sufficiently close to the junction to inhibit splicing out of the intervening exon during processing of precursor mRNA to mature mRNA (e.g., with the 3' or 5' 5 terminus of the antisense oligonucleotide being positioned within about, for example, 10, 5, 3, or 2 nucleotides of the intron/exon junction).

When practicing the present invention, the antisense nucleotides administered may be related in 10 origin to the species to which it is administered. When treating humans, human antisense may be used if desired.

Pharmaceutical compositions comprising an antisense oligonucleotide as given above effective to reduce expression of an A<sub>1</sub> or A<sub>2</sub> adenosine receptor by 15 passing through a cell membrane and binding specifically with mRNA encoding an A<sub>1</sub> or A<sub>2</sub> adenosine receptor in the cell so as to prevent its translation are another aspect of the present invention. Such compositions are provided in a suitable pharmaceutically acceptable carrier (e.g., 20 sterile pyrogen-free saline solution). The antisense oligonucleotides may be formulated with a hydrophobic carrier capable of passing through a cell membrane (e.g., in a liposome, with the liposomes carried in a pharmaceutically acceptable aqueous carrier). The 25 oligonucleotides may also be coupled to a substance which inactivates mRNA, such as a ribozyme. Such oligonucleotides may be administered to a subject to inhibit the activation of A<sub>1</sub> or A<sub>2</sub> adenosine receptors, which subject is in need of such treatment for any of the 30 reasons discussed herein. Furthermore, the pharmaceutical formulation may also contain chimeric molecules comprising antisense oligonucleotides attached to molecules which are known to be internalized by cells. These oligonucleotide conjugates utilize cellular uptake 35 pathways to increase cellular concentrations of oligonucleotides. Examples of macromolecules used in this manner include transferrin, asialoglycoprotein

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(bound to oligonucleotides via polylysine) and streptavidin.

In the pharmaceutical formulation the antisense compound may be contained within a lipid particle or 5 vesicle, such as a liposome or microcrystal. The particles may be of any suitable structure, such as unilamellar or plurilamellar, so long as the antisense oligonucleotide is contained therein. Positively charged 10 lipids such as N-[1-(2,3-dioleoyloxi)propyl]-N,N,N-trimethyl-ammoniummethylsulfate, or "DOTAP," are particularly preferred for such particles and vesicles. The preparation of such lipid particles is well known. See, e.g., U.S. Patent Nos. 4,880,635 to Janoff et al.; 4,906,477 to Kurono et al.; 4,911,928 to Wallach; 15 4,917,951 to Wallach; 4,920,016 to Allen et al.; 4,921,757 to Wheatley et al.; etc.

Subjects may be administered the active composition by any means which transports the antisense nucleotide composition to the lung. The antisense 20 compounds disclosed herein may be administered to the lungs of a patient by any suitable means, but are preferably administered by generating an aerosol comprised of respirable particles, the respirable particles comprised of the antisense compound, which 25 particles the subject inhales. The respirable particles may be liquid or solid. The particles may optionally contain other therapeutic ingredients.

Particles comprised of antisense compound for practicing the present invention should include particles 30 of respirable size: that is, particles of a size sufficiently small to pass through the mouth and larynx upon inhalation and into the bronchi and alveoli of the lungs. In general, particles ranging from about .5 to 10 microns in size are respirable. Particles of non- 35 respirable size which are included in the aerosol tend to deposit in the throat and be swallowed, and the quantity of non-respirable particles in the aerosol is preferably

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minimized. For nasal administration, a particle size in the range of 10-500  $\mu\text{m}$  is preferred to ensure retention in the nasal cavity.

Liquid pharmaceutical compositions of active compound for producing an aerosol can be prepared by combining the antisense compound with a suitable vehicle, such as sterile pyrogen free water. Other therapeutic compounds may optionally be included.

Solid particulate compositions containing respirable dry particles of micronized antisense compound may be prepared by grinding dry antisense compound with a mortar and pestle, and then passing the micronized composition through a 400 mesh screen to break up or separate out large agglomerates. A solid particulate composition comprised of the antisense compound may optionally contain a dispersant which serves to facilitate the formation of an aerosol. A suitable dispersant is lactose, which may be blended with the antisense compound in any suitable ratio (e.g., a 1 to 1 ratio by weight). Again, other therapeutic compounds may also be included.

The dosage of the antisense compound administered will depend upon the disease being treated, the condition of the subject, the particular formulation, the route of administration, the timing of administration to a subject, etc. In general, intracellular concentrations of the oligonucleotide of from .05 to 50  $\mu\text{M}$ , or more particularly .2 to 5  $\mu\text{M}$ , are desired. For administration to a subject such as a human, a dosage of from about .01, .1, or 1 mg/Kg up to 50, 100, or 150 mg/Kg or more is typically employed. Depending on the solubility of the particular formulation of active compound administered, the daily dose may be divided among one or several unit dose administrations. Administration of the antisense compounds may be carried out therapeutically (i.e., as a rescue treatment) or prophylactically.

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Aerosols of liquid particles comprising the antisense compound may be produced by any suitable means, such as with a nebulizer. See, e.g., U.S. Patent No. 4,501,729. Nebulizers are commercially available devices 5 which transform solutions or suspensions of the active ingredient into a therapeutic aerosol mist either by means of acceleration of a compressed gas, typically air or oxygen, through a narrow venturi orifice or by means of ultrasonic agitation. Suitable formulations for use 10 in nebulizers consist of the active ingredient in a liquid carrier, the active ingredient comprising up to 40% w/w of the formulation, but preferably less than 20% w/w. The carrier is typically water or a dilute aqueous alcoholic solution, preferably made isotonic with body 15 fluids by the addition of, for example, sodium chloride. Optional additives include preservatives if the formulation is not prepared sterile, for example, methyl hydroxybenzoate, antioxidants, flavoring agents, volatile oils, buffering agents and surfactants.

20           Aerosols of solid particles comprising the active compound may likewise be produced with any solid particulate medicament aerosol generator. Aerosol generators for administering solid particulate medicaments to a subject produce particles which are 25 respirable, as explained above, and generate a volume of aerosol containing a predetermined metered dose of a medicament at a rate suitable for human administration. One illustrative type of solid particulate aerosol generator is an insufflator. Suitable formulations for 30 administration by insufflation include finely comminuted powders which may be delivered by means of an insufflator or taken into the nasal cavity in the manner of a snuff. In the insufflator, the powder (e.g., a metered dose thereof effective to carry out the treatments described 35 herein) is contained in capsules or cartridges, typically made of gelatin or plastic, which are either pierced or opened in situ and the powder delivered by air drawn

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through the device upon inhalation or by means of a manually-operated pump. The powder employed in the insufflator consists either solely of the active ingredient or of a powder blend comprising the active 5 ingredient, a suitable powder diluent, such as lactose, and an optional surfactant. The active ingredient typically comprises from 0.1 to 100 w/w of the formulation. A second type of illustrative aerosol generator comprises a metered dose inhaler. Metered dose 10 inhalers are pressurized aerosol dispensers, typically containing a suspension or solution formulation of the active ingredient in a liquified propellant. During use these devices discharge the formulation through a valve adapted to deliver a metered volume, typically from 10 to 15 15  $\mu\text{l}$ , to produce a fine particle spray containing the active ingredient. Suitable propellants include certain chlorofluorocarbon compounds, for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane and mixtures thereof. The 20 formulation may additionally contain one or more co-solvents, for example, ethanol, surfactants, such as oleic acid or sorbitan trioleate, antioxidants and suitable flavoring agents.

The aerosol, whether formed from solid or 25 liquid particles, may be produced by the aerosol generator at a rate of from about 10 to 150 liters per minute, more preferably from about 30 to 150 liters per minute, and most preferably about 60 liters per minute. Aerosols containing greater amounts of medicament may be 30 administered more rapidly.

The following examples are provided to illustrate the present invention, and should not be construed as limiting thereon. In these examples,  $\mu\text{M}$  means micromolar,  $\text{mL}$  means milliliters,  $\mu\text{m}$  means 35 micrometers,  $\text{mm}$  means millimeters,  $\text{cm}$  means centimeters,  $^{\circ}\text{C}$  means degrees Celsius,  $\mu\text{g}$  means micrograms,  $\text{mg}$  means

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milligrams, g means grams, kg means kilograms, M means molar, and h means hours.

**EXAMPLE 1**

Design and synthesis of antisense oligonucleotides

5        The design of antisense oligonucleotides against the A<sub>1</sub> and A<sub>2</sub> adenosine receptors may require the solution of the complex secondary structure of the target A<sub>1</sub> receptor mRNA and the target A<sub>2</sub> receptor mRNA. After generating this structure, antisense nucleotides are  
10      designed which target regions of mRNA which might be construed to confer functional activity or stability to the mRNA and which optimally may overlap the initiation codon. Other target sites are readily usable. As a demonstration of specificity of the antisense effect,  
15      other oligonucleotides not totally complementary to the target mRNA, but containing identical nucleotide compositions on a w/w basis, are included as controls in antisense experiments.

Adenosine A<sub>1</sub> receptor mRNA secondary structure  
20      was analyzed and used as described above to design a phosphorothioate antisense oligonucleotide. The antisense oligonucleotide which was synthesized was designated HAdA1AS and had the following sequence:

5'-GAT GGA GGG CGG CAT GGC GGG-3' (SEQ ID NO:1)

25        As a control, a mismatched phosphorothioate antisense nucleotide designated HAdA1MM was synthesized with the following sequence:

5'-GTA GCA GGC GGG GAT GGG GGC-3' (SEQ ID NO:2)

30        Each oligonucleotide had identical base content and general sequence structure. Homology searches in GENBANK (release 85.0) and EMBL (release 40.0) indicated that the antisense oligonucleotide was specific for the human and rabbit adenosine A<sub>1</sub> receptor genes, and that the

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mismatched control was not a candidate for hybridization with any known gene sequence.

Adenosine A<sub>1</sub> receptor mRNA secondary structure was similarly analyzed and used as described above to 5 design two phosphorothioate antisense oligonucleotides. The first antisense oligonucleotide (HAdA3AS1) synthesized had the following sequence:

5'-GTT GTT GGG CAT CTT GCC-3' (SEQ ID NO:3)

As a control, a mismatched phosphorothioate antisense 10 oligonucleotide (HAdA3MM1) was synthesized, having the following sequence:

5'-GTA CTT GCG GAT CTA GGC-3' (SEQ ID NO:4)

A second phosphorothioate antisense oligonucleotide (HAdA3AS2) was also designed and 15 synthesized, having the following sequence:

5'-GTG GGC CTA GCT CTC GCC-3' (SEQ ID NO:5)

Its control oligonucleotide (HAdA3MM2) had the sequence:

5'-GTC GGG GTA CCT GTC GGC-3' (SEQ ID NO:6)

Phosphorothioate oligonucleotides were 20 synthesized on an Applied Biosystems Model 396 Oligonucleotide Synthesizer, and purified using NENSORB chromatography (DuPont, MD).

#### EXAMPLE 2

##### Testing of A<sub>1</sub>-Adenosine Receptor

##### Antisense Oligonucleotides in vitro

The antisense oligonucleotide against the human A<sub>1</sub> receptor (SEQ ID NO:1) described above was tested for

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efficacy in an in vitro model utilizing lung adenocarcinoma cells HTB-54. HTB-54 lung adenocarcinoma cells were demonstrated to express the A<sub>1</sub> adenosine receptor using standard northern blotting procedures and 5 receptor probes designed and synthesized in the laboratory.

HTB-54 human lung adenocarcinoma cells (106/100 mm tissue culture dish) were exposed to 5.0  $\mu$ M HAdA1AS or HAdA1MM for 24 hours, with a fresh change of media and 10 oligonucleotides after 12 hours of incubation. Following 24 hour exposure to the oligonucleotides, cells were harvested and their RNA extracted by standard procedures. A 21-mer probe corresponding to the region of mRNA targeted by the antisense (and therefore having the same 15 sequence as the antisense, but not phosphorothioated) was synthesized and used to probe northern blots of RNA prepared from HAdA1AS-treated, HAdA1MM-treated and non-treated HTB-54 cells. These blots showed clearly that HAdA1AS but not HAdA1MM effectively reduced human 20 adenine receptor mRNA by >50%. This result showed that HAdA1AS is a good candidate for an anti-asthma drug since it depletes intracellular mRNA for the adenosine A<sub>1</sub> receptor, which is involved in asthma.

#### EXAMPLE 3

25 Efficacy of A<sub>1</sub>-Adenosine Receptor  
Antisense Oligonucleotides in vivo

A fortuitous homology between the rabbit and human DNA sequences within the adenosine A<sub>1</sub> gene overlapping the initiation codon permitted the use of the 30 phosphorothioate antisense oligonucleotides initially designed for use against the human adenosine A<sub>1</sub> receptor in a rabbit model.

Neonatal New Zealand white Pasteurella-free rabbits were immunized intraperitoneally within 24 hours 35 of birth with 312 antigen units/mL house dustmite (*D. farinae*) extract (Berkeley Biologicals, Berkeley, CA),

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mixed with 10% kaolin. Immunizations were repeated weekly for the first month and then biweekly for the next 2 months. At 3-4 months of age, eight sensitized rabbits were anesthetized and relaxed with a mixture of ketamine 5 hydrochloride (44 mg/kg) and acepromazine maleate (0.4 mg/kg) administered intramuscularly.

The rabbits were then laid supine in a comfortable position on a small molded, padded animal board and intubated with a 4.0-mm intratracheal tube 10 (Mallinkrodt, Inc., Glens Falls, NY). A polyethylene catheter of external diameter 2.4 mm with an attached latex balloon was passed into the esophagus and maintained at the same distance (approximately 16 cm) from the mouth throughout the experiments. The 15 intratracheal tube was attached to a heated Fleisch pneumotachograph (size 00; DOM Medical, Richmond, VA), and flow was measured using a Validyne differential pressure transducer (Model DP-45161927; Validyne Engineering Corp., Northridge, CA) driven by a Gould 20 carrier amplifier (Model 11-4113; Gould Electronic, Cleveland, OH). The esophageal balloon was attached to one side of the differential pressure transducer, and the outflow of the intratracheal tube was connected to the opposite side of the pressure transducer to allow 25 recording of transpulmonary pressure. Flow was integrated to give a continuous tidal volume, and measurements of total lung resistance (RL) and dynamic compliance (Cdyn) were calculated at isovolumetric and flow zero points, respectively, using an automated 30 respiratory analyzer (Model 6; Buxco, Sharon, CT).

Animals were randomized and on Day 1 pretreatment values for PC<sub>50</sub> were obtained for aerosolized adenosine. Antisense (HAdA1AS) or mismatched control (HAdA1MM) oligonucleotides were dissolved in 35 sterile physiological saline at a concentration of 5000 ug (5 mg) per 1.0 ml. Animals were subsequently administered the aerosolized antisense or mismatch

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oligonucleotide via the intratracheal tube (approximately 5000  $\mu$ g in a volume of 1.0 ml), twice daily for two days.

Aerosols of either saline, adenosine, or antisense or mismatch oligonucleotides were generated by an ultrasonic 5 nebulizer (DeVilbiss, Somerset, PA), producing aerosol droplets 80% of which were smaller than 5  $\mu$ m in diameter.

In the first arm of the experiment, four randomly selected allergic rabbits were administered antisense oligonucleotide and four the mismatched control 10 oligonucleotide. On the morning of the third day, PC50 values (the concentration of aerosolized adenosine in mg/ml required to reduce the dynamic compliance of the bronchial airway 50% from the baseline value) were obtained and compared to PC50 values obtained for these 15 animals prior to exposure to oligonucleotide.

Following a 1 week interval, animals were crossed over, with those previously administered mismatch control oligonucleotide now administered antisense oligonucleotide, and those previously treated with 20 antisense oligonucleotide now administered mismatch control oligonucleotide. Treatment methods and measurements were identical to those employed in the first arm of the experiment. It should be noted that in 25 six of the eight animals treated with antisense oligonucleotide, adenosine-induced bronchoconstriction could not be obtained up to the limit of solubility of adenosine, 20 mg/ml. For the purpose of calculation, PC50 values for these animals were set at 20 mg/ml. The values given therefore represent a minimum figure for 30 antisense effectiveness. Actual effectiveness was higher. The results of this experiment are illustrated in both Figure 1 and Table 1.

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TABLE 1. EFFECTS OF ADENOSINE A<sub>1</sub> RECEPTOR ANTISENSE OLIGONUCLEOTIDE UPON PC50 VALUES IN ASTHMATIC RABBITS.

5	Mismatch Control		A <sub>1</sub> receptor Antisense oligonucleotide	
	Pre oligonucleotide	Post oligonucleotide	Pre oligonucleotide	Post oligonucleotide
	3.56 ± 1.02	5.16 ± 1.93	2.36 ± 0.68	>19.5 ± 0.34**

Results are presented as the mean (N = 8) ± SEM. Significance was determined by repeated-measures analysis of variance (ANOVA), and Tukey's protected t test. \*\*Significantly different from all other groups, P < 0.01.

10 In both arms of the experiment, animals receiving the antisense oligonucleotide showed an order of magnitude increase in the dose of aerosolized adenosine required to reduce dynamic compliance of the lung by 50%. No effect of the mismatched control 15 oligonucleotide upon PC50 values was observed. No toxicity was observed in any animal receiving either antisense or control inhaled oligonucleotide.

These results show clearly that the lung has exceptional potential as a target for antisense 20 oligonucleotide-based therapeutic intervention in lung disease. They further show, in a model system which closely resembles human asthma, that downregulation of the adenosine A<sub>1</sub> receptor largely eliminates adenosine-induced bronchoconstriction in asthmatic airways. 25 Bronchial hyperresponsiveness in the allergic rabbit model of human asthma is an excellent endpoint for antisense intervention since the tissues involved in this response lie near to the point of contact with aerosolized oligonucleotides, and the model closely 30 simulates an important human disease.

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**EXAMPLE 4**

Specificity of A<sub>1</sub>-adenosine receptor  
Antisense oligonucleotide

At the conclusion of the crossover experiment 5 of Example 3, airway muscle from all rabbits was quantitatively analyzed for adenosine A<sub>1</sub> receptor number. As a control for the specificity of the antisense oligonucleotide, adenosine A<sub>2</sub> receptors, which should not have been affected, were also quantified.

10 Airway smooth muscle tissue was dissected from each rabbit and a membrane fraction prepared according to described methods (J. Kleinstein and H. Glossmann, *Naunyn-Schmiedeberg's Arch. Pharmacol.* 305, 191-200 (1978), with slight modifications. Crude plasma membrane 15 preparations were stored at - 70°C until the time of assay. Protein content was determined by the method of Bradford (M. Bradford, *Anal. Biochem.* 72, 240-254 (1976)). Frozen plasma membranes were thawed at room temperature and were incubated with 0.2 U/ml adenosine 20 deaminase for 30 minutes at 37°C to remove endogenous adenosine. The binding of [<sup>3</sup>H]DPCPX (A<sub>1</sub> receptor-specific) or [<sup>3</sup>H]CGS-21680 (A<sub>2</sub> receptor-specific) was measured as previously described. S. Ali et al., *J. Pharmacol. Exp. Ther.* 268, 1328-1334 (1994); S. Ali et 25 al., *Am. J. Physiol* 266, L271-277 (1994).

As illustrated in both Figure 2 and Table 2, animals treated with adenosine A<sub>1</sub> antisense oligonucleotide in the crossover experiment had a nearly 75% decrease in A<sub>1</sub> receptor number compared to controls, 30 as assayed by specific binding of the A<sub>1</sub>-specific antagonist DPCPX. There was no change in adenosine A<sub>2</sub> receptor number, as assayed by specific binding of the A<sub>2</sub> receptor-specific agonist 2-[p-(2-carboxyethyl)-phenethylamino]-5'-(N-ethylcarboxamido) adenosine (CGS- 35 21680).

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TABLE 2. SPECIFICITY OF ACTION OF ADENOSINE A<sub>1</sub> RECEPTOR ANTISENSE OLIGONUCLEOTIDE.

	Mismatch Control oligonucleotide	A <sub>1</sub> Antisense oligonucleotide
5	A <sub>1</sub> -Specific Binding	1105 ± 48** 293 ± 18
	A <sub>2</sub> -Specific Binding	302 ± 22 442 ± 171

Results are presented as the mean (N = 8) ± SEM. Significance was determined by repeated-measures analysis of variance (ANOVA), and Tukey's protected t test. \*\*Significantly different from mismatch control, P < 0.01.

10 The above demonstrates the effectiveness of antisense oligonucleotides in treating airway diseases. Since the antisense oligonucleotides described above eliminate the receptor systems responsible for adenosine-mediated bronchoconstriction, it may be less imperative to  
 15 eliminate adenosine from them. However, it would be preferable to eliminate adenosine from even these oligonucleotides. Examples of such adenosine-free oligonucleotides are provided below in Example 5.

#### EXAMPLE 5

20 The method of the present invention is also practiced with the following antisense oligonucleotides targeted to their corresponding proteins, in essentially the same manner as given above, for the treatment of various conditions in the lungs. Described below is a  
 25 series of antisense oligonucleotides targetting the mRNA of proteins involved in inflammation. Adenosine has been eliminated from their nucleotide content to prevent its liberation during degradation.

In the following, the first sequence provided  
 30 after the name of the targeted inflammation-involved protein is the antisense sequence that targets the initiation codon, wherein the naturally-occurring adenosine is substituted by one of the following: (1) a universal base that is not adenosine; (2) a adenosine  
 35 analog that lacks the ability to bind to the adenosine A1

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and/or A3 receptors; or (3) a "spacer." Any one of these three is represented in the sequence as the letter "B," recognized by the IUPAC-IUB Nomenclature Commission as "not-A." See *Patentin User Manual*, p.99 (November 1990).  
5 Listed following the antisense sequence targeted against the initiation codon are additional antisense oligonucleotide sequences directed against other portions of the mRNA of the targeted protein. These additional sequences are the "des-adenosine antisense sequences," in  
10 that they do not contain adenosine within the sequence.

15 Fragments of the following sequences that are at least ten, and more preferably at least twelve, nucleotides in length are also an aspect of the present invention and are useful in carrying out the present invention. Fragments set forth below that span multiple lines of text indicate "5'" at the beginning thereof, and "-3'" at the end thereof.

**Human A1 adenosine receptor:**

20 5'-GGC GGC CTG GBB BGC TGB GBT GGB GGG CGG CBT  
GGC GGG CBC BGG CTG GGC-3'

**des-adenosine antisense sequences:**  
TTT TCC TTC CTT TGT CTC TCT TC

GCT CCC GGC TGC CTG

CTC GGC CGT GCG GCT CTG TCG CTC CCG GT

25 CCG CCG CCC TCC GGG GGG TC

TGC TGC CGT TGG CTG CCC

CTT CTG CGG GTC GCC GG

TGC TGG GCT TGT GGC

GGC CTC TCT TCT GGG

30 CCT GGT CCC TCC GT

GGT GGC TCC TCT GC

GCT TGG TCC TGG GGC TGC

TGC TCT CCT CTC CTT

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**Human A2a adenosine receptor:**

GTBCBCCGBGGBGCCCBTGBTGGGCBTGCCCBGBCGBCBGGC

*des-adenosine antisense sequences:*

HSA2ARECAS1: TGC TTT TCT TTT CTG GGC CTC (SEQ ID NO:7)

HSA2ARECAS2: TGT GGT CTG TTT TTT TCT G

HSA2ARECAS3: GCC CTG CTG GGG CGC TCT CC

HSA2ARECAS4: GCC GCC CGC CTG GCT CCC

HSA2ARECAS5: GGB GCC CBT GBT GGG CBT GCC

HSA2ARECAS6: GTG GTT CTT GCC CTC CTT TGG CTG

HSA2ARECAS7: CCG TGC CCG CTC CCC GGC

HSA2ARECAS8: CTC CTG GCG GGT GGC CGT TG

HSA2ARECAS9: GGC CCG TGT TCC CCT GGG

HSA2ARECAS10: GCC TGG GGC TCC CTT CTC TC

HSA2ARECAS11: GCC CTT CTT GCT GGG CCT C

HSA2ARECAS12: TGC TGC TGC TGG TGC TGT GGC CCCC

**Human A2b adenosine receptor:**5'-BCBGGCGTCCCTGTGTCTCCBGCGBGBTGGCC  
GGGCCBGCCTGGGCCCC-3'*des-adenosine antisense sequences:*HSA2BRECAS1: 5'-GGC GCC GTG CCG CGT CTT GGT GGC  
GGC GG-3' (SEQ ID NO:8)HSA2BRECAS2: 5'-GTT CGC GCC CGC GCG GGG CCC CTC  
CGG TCC-3'HSA2BRECAS3: 5'-TTG GCC CGC GCG CCC GCC CGT CTC  
GGG CTG GGC GG-3'

HSA2BRECAS4: CGG GTC GGG GCC CCC CGC GGC C

HSA2BRECAS5: 5'-GCC TCG GGG CTG GGG CGC TGG TGG  
CGG GG-3'

HSA2BRECAS6: CCG CGC CTC CGC CTG CCG CTT CTG

HSA2BRECAS7: GCT GGG CCC CGG GCG CCC CCT

HSA2BRECAS8: CCC CTC TTG CTC GGG TCC CCG TG

**Human A3 adenosine receptor**5'-BCB GBG CBG TGC TGT TGT TGG GCB TCT TGC CTT  
CCC BGG G-3'*des-adenosine antisense oligonucleotides:*  
CCC TTT TCT GGT GGG GTG

GTG CTG TTG TTG GGC

TTT CTT CTG TTC CC

**40 Human IgE receptor  $\beta$ :**5'-BTTTGCTCTCCTBTTBCTTTCTGTGTCCBTTTTT  
CBTTBBCGGBGCTGT-3'*des-adenosine antisense sequences:*HUMIgE $\beta$ rAS1: TTT CCC CTG GGT CTT CC (SEQ ID NO:9)HUMIgE $\beta$ rAS2: CTC CTG CTC TTT TTT C

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**Human Fc-epsilon receptor CD23 antigen (IgE receptor):**  
 5' -TCTCTGBBTBTTGBCCTTCCTCCBTGGCGGTCTGCTT  
 GGBTTCTCCGB-3'

5 **des-adenosine antisense sequences:**  
 HUMIgErCD23AS1: GCC TGT GTC TGT CCT CCT (SEQ ID NO:10)  
 HUMIgErCD23AS2: GCT TCG TTC CTC TCG TTC  
 HUMIgErCD23AS3: CTG CTT GGT GCC CTT GCC G  
 HUMIgErCD23AS4: GTC CTG CTC CTC CGG GCT GTG G  
 10 HUMIgErCD23AS5: 5'-GTC GTG GCC CTG GCT CCG GCTGGT GGG CTC CCC TGG-3'  
 HUMIgErCD23AS6: CCT TCG CTG GCT GGC GGC GTG C  
 HUMIgErCD23AS7: GGG TCT TGC TCT GGG CCT GGC TGT  
 HUMIgErCD23AS8: GGC CGT GGT TGG GGG TCT TC  
 15 HUMIgErCD23AS9: GCT GCC TCC GTT TGG GTG GC

**Human IgE receptor,  $\alpha$  subunit:**  
 5' -BCBGTBGBGTBGGGGBTTCCTGGCBGGBGCCBTC  
 TTCTTCBTGGBCTCC-3'

and

20 5' -TTC BBG GBG BCC TTB GGT TTC TGB GGG BCT GCT  
 BBC BCG CCB TCT GGB GC-3'

**des-adenosine antisense sequences:**  
 HUMIgEr $\alpha$ AS1: GCCTTCCTGGTTCTCTT (SEQ ID NO:11)

GTT GTT TTT GGG GTT TGG CTT

25 **Human IgE receptor, Fc epsilon R:**  
 5' -GBT CTC TGB BTB TTGB CCT TCC BTG GCG GTC CTG  
 CTT GGB-3'

30 **des-adenosine antisense sequences:**  
 HSJGEBFRAS1: GCC TGT GTC TGT CCT CCT (SEQ ID NO:12)  
 HSJGEBFRAS2: GCT TCG TTC CTC TCG TTC  
 HSJGEBFRAS3: CTG CTT GGT GCC CTT GCC G  
 HSJGEBFRAS4: GTC CTG CTC CTC CGG GCT GTG G  
 35 HSJGEBFRAS5: 5'-GTC CTC GCC CTG GCT CCG GCT GGT  
 GGG CTC CCC TGG-3'  
 HSJGEBFRAS6: CCT TCG CTG GCT GGC GGC GTG C  
 HSJGEBFRAS7: CCC BGB BCG BGB CCC GGB CCG BCB  
 HSJGEBFRAS8: GGC CGT GGT TGG GGG TCT TC  
 HSJGEBFRAS9: GCT GCC TCC GTT TGG GTG GC

40 **Human histidine decarboxylase:**  
 5' -CTC TGT CCC TCT CTC TCT GTB CTC CTC BGG CTC  
 CBT CBT CTC CCT TGG GC-3'

**des-adenosine antisense sequences:**

45 HUMHDCAS1: TCT CCC TTG GGC TCT GGC TCC TTC TC  
 (SEQ ID NO:13)

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HUMHDCAS2: TCT CTC TCC CTC TCT CTC TGT  
 HUMHDCAS3: CGCCTCCGCCCTGGCTGCTGGGGTGGTGGTGC  
 HUMHDCAS4: TTT TGT TCT TCC TTG CTG CC  
 HUMHDCAS5: GCC CCG CTG CTT GTC TTC CTC G

5 Human beta tryptase:

5'-GGG CCT GGC CTG GGG CBG GGG CCG CGT BGG CGC  
 GGC TCG CCB GGB CGG GCB GCG CCB GCB GCB GCB GBT  
 TCB GCB TCC TGG-3'

*des-adenosine antisense sequences:*

10 HUMBTRYPAS1: CTTGCTCCTGGGGCCTCCTG (SEQ ID  
 NO:14)  
 HUMBTRYPAS2: GTC CCT CCG GGT GTT CCC GGC

**Human tryptase-I:**

15 5'-CCT GGB CTG GGG CBG GGG CCG CGT BGG CGC GGC  
 TCG CCB GGB CGG GCB GCG CCB GCB GCB GCB GGC TCB  
 GCB TCC TGG CCB CGG BBT TCC-3'

*des-adenosine antisense sequences:*

HUMTRYAS1: CTTGCTCCTGGGGCCTCCTG (SEQ ID NO:15)  
 HUMTRYAS2: GTC CCT CTG GCT G TT CCC GGC

20 Human prostaglandin D synthase:

5'-CCC CBG CBG GBC CBG TCC CBT CCB CBG CGT GTG  
 BTG BGT BGC CBT TCT CCT GCB GCC GBG-3'

*des-adenosine antisense sequences:*

25 HUMPROSYNAS1: GGTGTGCGGGGGCTGGTGCC (SEQ ID NO:16)  
 HUMPROSYNAS 2: CCT GGG CCT CGG GTG CTG CCT GT  
 HUMPROSYNAS 3: GCG CTG CCT TCT TCT CCT GG  
 HUMPROSYNAS 4: 5'-GTC CTC GCC GGG GCC CTT GCT  
 GCC CTG GCT GT -3'  
 HUMPROSYNAS 5: GCC CTG GGG GTC TGG GTT CGGCTGT

30 Human cyclooxygenase-2:

5'-TGB GCG CCB GGB CCG CGC BCB GCB GCB GGG CGC  
 GGG CGB GCB TCG CBG CGG CGG GCB GGG-3'

*des-adenosine antisense sequences:*

35 HUMCYCLOXAS1: GGGCGCGGGCGBGCCTCGC (SEQ ID NO:17)  
 HUMCYCLOXAS2: TTT GGG CTT TTC TCC TTT GGT T

**Human eosinophil cationic protein:**

5'-CBG BCB BBT TTG GGB BGT GBB CBG TTT TGG BBC  
 CBT GTT TCC CBG TCT CTG BGC TGT GGC-3'

*des-adenosine antisense sequences:*

40 HSECPAS1: CCTCCTTCC TGG TCT GTC TGC (SEQ ID  
 NO:18)

**Human eosinophil derived neurotoxin:**

5'-CCC CBB CBG BBG BBG CBG BCB BBT TTG GGB BGT  
 GBB CBG TTT TGG BBC CBT GTT TCC TGT-3'

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5                   *des-adenosine antisense sequences:*  
 HSEOSDNAS1: GCC CTG CTG CTC TTT CTG CT (SEQ ID  
 NO:19)

HSEOSDNAS 2: TCC CTT GGT GGG TTG GGC C  
 HSEOSDNAS 3: GCT GGT TGT TCT GGG GTT C  
 HSEOSDNAS 4: TTG CTG CCC CTT CTG TCC C  
 HSEOSDNAS 5: TGT TTG CTG GTG TCT GCG C

10                  *Human eosinophil major basic protein:*  
 GGG GGB GTT TCB TCT TGG CTT T

15                  *des-adenosine antisense sequences:*  
 TCT CCC CTT GTT CCT CCC C

TCT CCT GCT CTG GTG TCT CCT C

TTC CCT CCC TCC CCT GCC

GTG TTG TCT GTG GGT GTC C

20                  GTT TCG CTC TTG TTG CCC  
 TGG GCC CTT CCC TGC TGG

25                  *Human eosinophil peroxidase:*  
 5'-GCB CCG TCC BGT GBT GGT GCG GTB CTT GTC GCT  
 GCB GCG CTC GGC CTG GTC CCG GBG BGC-3'

30                  *des-adenosine antisense sequences:*  
 HSEPAS1: GCGCTCGGCCTGGTCCCGG (SEQ ID NO:20)  
 HSEPAS2: GGG TCT CCT CTT GTT GTT GC  
 HSEPAS3: TTG CGC CTC CTG CTG GGG GT CC  
 HSEPAS4: CTC TGT TCT TGT TTT GGG GGC  
 HSEPAS5: GGG CCC GGC CGT TGT CTT G  
 HSEPAS6: GTT TGG GGG TTT CCG TTG  
 HSEPAS7: GGG TTC TCC TGG CCC GGG CCT TGC CC  
 HSEPAS8: GGC CGT GGT CCC GGC TTC GTT GC  
 HSEPAS9: CCT GTC TCC GTC TCG GCT CTT CTG  
 HSEPAS10: GGG CCT TGC GCT GTC TTT GGT G

35                  *Human intercellular adhesion molecule-1 (CAM-1):*

40                  5'- CGG BGC CTC CCC GGG GCB GGB TGB CTT TTG BGG  
 GGG BCB CBG BTG TCT GGG CBT TGC CBG GTC CTG GGB  
 BCB GBG CCC CGB GCB GGB CCB GGB GTG CGG GCB GCG  
 CGG GCC GGG GGC TGC TGG GBG CCB TBG CGB GGC TGB  
 G-3'

45                  *des-adenosine antisense sequences:*  
 HSICAM1AS1: GCGCGGGCCGGGGCTGCTGGG (SEQ ID  
 NO:21)  
 HSICAM1AS2: GGT TGG CCC GGG GTG CCC C  
 HSICAM1AS3: GCC GCT GGG TGC CCT CGT CCTCTGCGGTC  
 HSICAM1AS4: GTG TCT CCT GGC TCT GGT TCC CC  
 HSICAM1AS5: 5'-GCT GCG CCC GTT GTC CTC TGG GGT  
 GGCCTTC-3'

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HSICAM1AS6: GCT CCC GGG TCT GGT TCT TGT GT  
 HSICAM1AS7: TGG GGG TCC CTT TTT GGG CCT GTT GT  
 HSICAM1AS8: GGC GTG GCT TGT GTG TTC GGT TTC  
 HSICAM1AS9: TGC CCT GTC CTC CGG CGT CCC

5 Human vascular cell adhesion molecule 1 (VCAM-1):  
 5'-CTG BGC BBG BTB TCT BGB TTC TGG GGT GGT CTC  
 GBT TTT BBBB GCT TGB GBB GCT GCB BBC BTT BTC  
 CBB BGT BTB TTT GBG GCT CCB BGG BTC BCG BCC BTC  
 TTC CCB GGC BTT TTG BGT TGC TGT CGT -3'

10 des-adenosine antisense sequences:  
 HSVCAM1AS1: CCTCTTTCTGTTTCCC (SEQ ID NO:22)  
 HSVCAM1AS2: CTC TGC CTT TGT TTG GGT TCG  
 HSVCAM1AS3: CTT CCT TTC TGC TTC TTC C  
 HSVCAM1AS4: CTGTGTCTCTGTCTCCGCTTTTCTTC  
 HSVCAM1AS5: GTC TTT GTT TTC TCT TCC TTG

Human endothelial leukocyte adhesion molecule (ELAM-1):  
 5'-BBG TGB GBG CTG BGB GBB BCT GTG BBG CBB TCB  
 TGB CTT CBB GBG TTC TTT TCB CCC -3'

20 des-adenosine antisense sequences:  
 HUMELAM1AAS1: GTTCTTGGCTTCTCTGTC (SEQ ID NO:23)  
 HUMELAM1AAS2: CGT TGG CTT CTC GTT GTC CC  
 HUMELAM1AAS3: TGT GGG CTT CTC GTT GTC CC  
 HUMELAM1AAS4: CCC TTC GGG GGC TGG TGG  
 HUMELAM1AAS5: GGC CGT CCT TGC CTG CTG G

25 Human P Selectin:  
 des-adenosine antisense sequences:  
 HUMPSELECTAS1: CTCTGCTGGT TTTCTGCCTT CTGCC  
 (SEQ ID NO:24)

30 Human endothelial monocyte activating factor:  
 des-adenosine antisense sequences:  
 HUMEMAPII1AS1: 5'-TTT TCT CTT TCG CTT TCT TTT  
 CGTCTCCTGTTCTCCTTTT-3' (SEQ ID  
 NO:25)  
 HUMEMAPII1AS2: 5'-TTG CTG TTT TTT CTC CTT CTT  
 CTC TCC TTT CTT TTC -3'

35 Human IL3:  
 5' -GGCGGBCCBGGBGTGGBGCBGGBGCBGGBCGGCB  
 GGC GGCTCCTGTTGGBTGGCBGGCBGGCBCTC -3'

40 des-adenosine antisense sequences:  
 HUMIL3AAS1: 5'-CTC TGT CTT GTT CTG GTC CTT CGT  
 GGG GCT CTG (SEQ ID NO:26) -3'  
 HUMIL3AAS2: TGT CGC GTG G GTG CGG CCG TGG CC

45 Human IL3 receptor:  
 5' -GCBGGGBCBGGGCBGGGCGBTGCBGGBGCBGCGT  
 GBGCCCBBGGBGGBCCTCGGGBBCGCBGCTCCG  
 GBBCGCBGGBCBGBGGTGCC-3'

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*des-adenosine antisense sequences:*  
 TCTGGGGTGTCTG

5                   GCCTTCGTGGTTCC

5                   TCTTCCTTCGTTGC

CGTCCCGGGGGCCCCCGGGCCT

GGCTGCGCTCCTGCCCGC

CTCTTCCCAGGCTCTT

10                  GCGCTGGGGGTGCTCC

CGTGTGTTGCGCCCTCCTCCTGGTCGC

GCTTGTCTTTGG

15                  GGCCGGCTTGCCCCGCCCTCCC

GGCGCCTGGCCCGGCC

TTCCTGGCTGCGTGCGC

20                  GTTCTGTTCTTCTTCCTGGC

**Human IL4:**

5' -GCCGGCCTGCTBGCBBGBCBGBGGGGGB  
 BGCBGTTGGGGGTGBGBCCCBTTBGGTGTGCB -3'

25

*des-adenosine antisense sequences:*  
 HUMIL4AS1: CTC TGG TTG GCT TCC TTC-3'  
 (SEQ ID NO:27)

**Human IL4 receptor:**

30

5' -GTTCCCBGBGCTTGCCBCCTGCBGCBGGBCCBGGCBGCTC  
 BCBGGGBBCBGGBGCCCBGBGCBBBGCCBCCCCBTGGGBG  
 BTGCCBGGCBCCBGGCTG-3'

*des-adenosine antisense sequences:*  
 TCTGCGCGCCCTGCTCC

35

CGCCCGGCTTCTCT

CGTGTGGGCTTCGG

40

CCCCGCGCTCCGTTGTTCTC

TGCTCGCTGGGCTTG

GGTTTCCTGGGGCCCTGGGTTTC

45

TCTGCCGGTCTGGTTTC

GGGTGCTGGCTGCG

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	CTTGGTGCTGGGCTCC
	GGCGGCTGCGGGCTGGGTTGGG
5	CTTGGCTGGTTCTGGCCTCGGG
	CCTCCCTCCTCCTCCTC
10	GCTCCCTTTTCTTCCTCT
	TCCCTGCTGCTCTC
	TGCCCTCCCTCCCTCCTGG
15	GGTGCCTCCTGGGCCCTGC
	GGCTGCTCCTGGCCCC
20	CTCTGGTCGGGCTGGC
	GGGGCGTCTCTGTGC
	CTGGCCTGGGTGCC
25	GCCTCTCCTGGGGG
	GGTGGCTCCCTGTCC
	CCTTTCCCCGGCTCC
30	GTGGGGCTTGGC
	GGGGGTCTGTGGCCTGCTCCTGGGG
35	AGGGGTCTGGGCCCTC
	TTTTGGGGGTCTGGCTTG
	GCCTGGCTGCCCTTCC
40	GGGGCCTGCCGTGGGGC
	TGTCCCTCTGTTGCTCCCCCT
45	TGCCTGCTGTCTGG
	GGTTCCCGCCTTCCCT
<b>Human IL5:</b>	
50	5' - GTGGGGBTTCCTGTGGGGBTGGCBTBCBCGTBGGCB GCTCCBBGBGCTBGCBBCTCBBBTGCBGBBGBCBTC CTCBTGGCTCTGBBBCG -3'



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GGGGCGGGGCGGGGCGGTGGCGGGC  
GGCGCTGCCCTGCGC  
5 GCGGCGCTGGCCCC  
TGCTGGCCGTGGCTGCGCGCTGCTGGCTGCCCT  
GCTGGCCGCGCCGGG  
10 GCCTGTCCGCCCTCTGCGGG  
CGCTGTCTCCTGGC  
TTGTCTTCCGGCTCT  
TCTGCTGGGGTGGG  
15 GCTGGGCGGCCGGCCCGGT  
GCTGGGGCTCCTCGGGGGG  
20 GGGGGCTCTTCCGG  
GCTGTCTCCCTCCGGG  
GCGGGGGTTCTGGCC  
25 GTGGGGGTCTTGCC  
TGGCCTCCGGGCTCC  
30 TGCTTGTCTGCCTTCCTTC  
TCTGGTCGGTTGTGGCTCG  
GGGCTCCGTGGGTCCCTGGC  
35 GCCCCGTTTGTGTTTGTC  
TTTCCCCCTGGCGT  
40 CCCTGTGCCCTCTCCTCTCCTCCTGCTTCTC  
GCTCTCCTTTGTGGG  
GCCCTCCCTGCTGCT  
45 CTTGGTTTGGGCT  
TTTTTTCTCTCCTCCTTTC  
50 GTGCGTGGGCCTCC

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**Human monocyte-derived neutrophil chemotactic factor:**

5                   5' - GGGGTGGBBBGGTTTGGBGTBTGTCCTTTBTGCBCTGB  
                  CBTCTBBGTTCTTBGCBCTCCTTGGCBBBCTGCB  
                  CTTCBCBCBGBGCTGCBGBBBTBCGGBBGGCTGCCB  
                  5            GBGBGCCBCGGCCBGGCTTGGBBGTCBTGTTBCBC  
                   BGTGBGBTGGTCTCCCTCCGG-3'

**des-adenosine antisense sequences:**

10                  HSMDNCFAS1: GCT TGT GTG CTC TGC TGT CTC T (SEQ  
                   ID NO:30)  
                   HSMDNCFAS2: 5'-TGG TTC CTT CCG GTG GTT TCT TCC  
                   TGG CTC TTG TCC T -3'  
                   HSMDNCFAS3: TTC TCT TGG CCC TTG GC

**Human neutrophil elastase (medullasin):**

15                  5' - GGGCTCCGCCGCGBGBGGTTBTGGGCTCCBGGBC  
                   CCGCBCCCGCGCCGBCGTTTBCBTTGCCBCGCBGTC  
                   GGCCGBCBTGBCGBBGTTGGCGCBTCBGGGTGGCG  
                   GCBGBBGTGGCTCCGCGCCTGCBGGGBCBCCBTGBB  
                   GGGCCBCGCGTGGGCGCGCTCGCCGGCCCCCB  
                   CTCCGBGGCCBGCCTGGCTGCCCBGCBGCBGGCCGG  
                   20           CBGGBCBCBGGCGBGGBCBGCCTGGCGGCBG  
                   GGTCBTGGTGGGGCTGGGCTCCGGGTCTCTGCC  
                   CGTGC-3'

**des-adenosine antisense oligonucleotides:**

25                  HSMEDURAS1: 5'-TGG TGG GGC TGG GGC TCC GGG GTC  
                   TCT GCC CCT CCG TGC-3' (SEQ ID NO:31)  
                   HSMEDURAS2: CGC GTG GGG CGG CGC TCG CCG GCCCCCCC  
                   HSMEDURAS3: CCT GCC GGG TGG GCT CCC GCC GCG  
                   HSMEDURAS4: CGC CGG CCT GCC GGC CCC TC  
                   HSMEDURAS5: 5'-GTG GGT CCT GCT GGC CGG GTC CGG  
                   GTC CCG GGG GTG GGG-3'  
                   HSMEDURAS6: CGC GBG TCG GCG GCC GBG GGT C

**Human neutrophil oxidase factor:**

35                  5' - CGGGBGTGGGGCTCTGGBCGGCBCTGBBGGCB  
                   CTCCCTTCBGBTCTCTGTGTCGCTGCCBGC  
                   BTTCCBGBGGCTGBTGGCTCCBCCBGGBC  
                   TBGBBBCTBGGBGGCC-3'

**des-adenosine antisense sequence:**

40                  HUMNOXFAS1: GGC CTC CBC CBG GGB CBT G (SEQ ID  
                   NO:32)  
                   HUMNOXFAS2: GTC CTT CTT GTC CGC TGC C  
                   HUMNOXFAS3: TCT CTG GGG TTT TCG GTC TGG GTG G  
                   HUMNOXFAS4: GCT TTC CTC CTG GGG CTG CTG CTG  
                   HUMNOXFAS5: 5'-GGC TCT TCT TTT TGT TTC TGG CCT  
                   GGTG-3'  
                   HUMNOXFAS6: CTC TCT CGT GCC CTT TCC  
                   HUMNOXFAS7: CTT GGG TGT CTT GTT TTT GT  
                   HUMNOXFAS8: 5' -GGCTCCBCCBGGBCBTGGTCCTTCTT  
                   GTCCGCTGCC -3'

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**Human cathepsin G:**

5' -CCCTCCBCBTCTGCTCTGBCCTGCTGGBCCTCTG  
 GBTCTGBBGBTBCGCCBTGTTBGGGGCGGGCGTG  
 GGGCCTGCTCTCCCGGCCCTCCGTTGTTCTCCCT  
 5 GOCTCBGCCCCBGTGGGTBGGBGBBBGGCCBGC  
 GBBGCBGGBGTGGCTGCBTCTTCCCTG -3'

**des-adenosine antisense sequences:**

HUMCTHGAS1: GTG GGG CCT GCT CTC CCG GCC TCC G  
 (SEQ ID NO:33)  
 10 HUMCTHGAS2: TGTGTTGCTGGGTGTTTCCGCTCTGG  
 HUMCTHGAS3: TCT GCC TTC GGG GGT CGT

**Human defensin 1:**

15 5' -CCGGGGCTGCBGCBCCCTCBTCBGCTCTTGCCT  
 GGBGTGGCTCBGCCCTGGGCTGCBGGGCCBCCB  
 GGBGBTGGCBGCBGGBTGGCGBGGGTCCCTCB  
 TGGCTGGGTCBCBGBTCCCTCTBGCTBGGCBGG  
 GTGBCCBGBGBGGGC-3'

**des-adenosine antisense sequences:**

20 HUMDEF1AAAS1: GGG TCC TCB TGG CTG GGG (SEQ ID  
 NO:34)  
 HUMDEF1AAAS2: GCC TGG GCC TGC BGG GCC  
 HUMDEF1AAAS3: GCT CTT GCC TGG BGT GGC TC  
 HUMDEF1AAAS4: GCC CBG BGT CTT CCC TGG T

**Human defensin 3:**

25 5' -CGCTGCBBTCTGCTCCGGGCTGCBGCBCCCTCBTC  
 BGCTCTGCTGGBGTGGCTCBGCTGGGCTGCBG  
 GGCCBCCBGGBGBTGGCBGCBGGBTGGCGBGGGT  
 CCTCBTGGCTGGGTCBCCTGGBGGBGGGBGCBGG-3'

**des-adenosine antisense sequences:**

30 HUMNTRIIIAS1: GGG TCC TCB TGG CTG GGG TC (SEQ  
 ID NO:35)  
 HUMNTRIIIAS2: CCT CTC TCC CGT CCT

**Human macrophage inflammatory protein-1-alpha: RANTES  
 RECEPTOR**

35 5' -GBGGGGCBGCBGTTGGGCCCBGGCCCTCTCGT  
 TCBCCTCTGGCBGGBGTGCBTCCCCBTBGTCB  
 BCTCTGTTGGTGGTGTCTCTGTGGTGTGTTG  
 GBGTTTCCBTCCCGCTTCTCTCTGGTTCCBBGGGB-3'

**des-adenosine antisense sequences:**

40 HUMRANTESAS1: GTC TTT GTT TCT GGG CTC GTG CC  
 (SEQ ID NO:36)  
 HUMRANTESAS2: CCB TCC CGG CTT CTC TCT GGT TCC  
 HUMRANTESAS3: GTC CTCTGT GGT GTT TGG  
 HUMRANTESAS4: 5' -CCC TGC TTC CTT TTG CCT GTT  
 45 TCTTTGTTT CTGGGCTCGT GCC -3'

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**RANTES:**

5                   5' - GGGCBBGGGCBGTGGCGGGCBTGTBGGC  
                   BBBGCBBGCBGGGTGTGGTGTCCGBGGBBTBTGGG  
                   GBGGCBGBTGCBGGCGCGBGBGGGCBGTBGCBB  
                   TGBGGGBTGBCBGCGBGGCGTGCCGCGGBGCCTTC  
                   BTGGTBCCGTGGBGBGGCTGTCGGBGG-3'

*des-adenosine antisense sequences:*

10                   GGGTGTGGTGTCCG  
                   CTTGGCGGTTCTTCGGGTG  
                   TTTCTTCTCTGGGTTGGC  
                   15                   CTGCTGCTCGTCGTGGTC  
                   GCTCCGCTCCCGGGTTC  
                   GTCTCGCTCTGTCGCC  
                   20                   CTTCCTCCTTGTC  
                   GTGTTCCCTCCCTTCCTTGCCCT

**Human muscarinic acetylcholine receptor HM1:**

25                   *des-adenosine antisense sequences:*  
                   HSHM1AS1: GTT CBT GGT GGC TBG GTG GGG C (SEQ ID  
                   NO:37)  
                   HSHM1AS2: GCT GCC CGG CGG GGT GTG CGC TTG GC  
                   HSHM1AS3: GCTCCCGTG CTC GGT TCT CTG TCTCCGGT  
                   30                   HSHM1AS4: CCC CCT TTG CCT GGC GTC TCG G  
                   HSHM1AS5: GCC TTC GTC CTC TTC CTC TTC TTG CTTCC  
                   HSHM1AS6: 5'-GCT CCG TGG GGG CTG CTTGGTGGG  
                   GCCCTG TGC CTC GGG GTC C-3'  
                   HSHM1AS7: CGG GGC TTC TGG CCC TTG CC

**35 Human muscarinic acetylcholine receptor HM3:**

*des-adenosine antisense sequences:*  
                   HSHM3AS1: GGG GTG GGT BGG CCG TGT CTG GGG (SEQ  
                   ID NO:38)  
                   HSHM3AS2: GTT GGC CBT GTT GGT TGC C  
                   40                   HSHM3AS3: TCT TGG TGG TGC GCC GGG C  
                   HSHM3AS4: 5'-GCG TCT TGG CTT TCT TCT CCT TCG  
                   GGC CCT CGG GCC GGT GCT TGT GG-3'  
                   HSHM3AS5: 5'-GCT CCT CCC GGG CGG CCT CCC CGG  
                   GCG GGG GCT TCT TG-3'  
                   45                   HSHM3AS6: GCG CTG GCG GGG GGG CCT CCT CC  
                   HSHM3AS7: 5'-GCT CTG TGG CTG GGC GTT CCT TGG  
                   TGT TCT GGG TGG C-3'  
                   HSHM3AS8: TGG CGG GCG TGG TGG CCT CTG TGG TGG  
                   HSHM3AS9: GGG CCC GCG GCT GCB GGG G  
                   50                   HSHM3AS10: TTG CCT GTC TGC TTC GTC  
                   HSHM3AS11: CTT TGC GCT CCC GGG CCG CC

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**Human fibronectin:***des-adenosine antisense sequences:*

5                    HUMFNA/HSFIB1AS1: CGG TTT CCT TTG CGG TC (SEQ ID NO:39)  
 HUMFNA/HSFIB1AS2: TTG GCC CGG GCT CCG GGT G  
 HUMFNA/HSFIB1AS3: CCC GCC CGC CCG CCG GCC GCCGC  
 HUMFNA/HSFIB1AS4: 5'-CCC GCC GGG CTG TCC CCG CCC CGC CCC-3'  
 HUMFNA/HSFIB1AS5: GGC CCG GGG CGC GGG GG  
 10                HUMFNA/HSFIB1AS6: CGG CCC TCC CGC CCC TCT GG  
 HUMFNA/HSFIB1AS7: GCC GGC GCG GGC GTC GG  
 HUMFNA/HSFIB1AS9: 5'-CCG CTC GCG CCT GGG GTT CCC TCT CCT CCCCCCTGTGC-3'  
 HUMFNA/HSFIB1AS10: GCC TGC CTC TTG CTC TTC  
 15                HUMFNA/HSFIB1AS11: TGC GTC CGC TGC CTT CTC CC  
 HUMFNA/HSFIB1AS12: CTC TCC TCG GCC GTT GCCTGTGC  
 HUMFNA/HSFIB1AS13: 5'-TGT CCG TCC TGT CGC CCT TCC GTG GTG C-3'  
 HUMFNA/HSFIB1AS14: TGT TGT CTC TTC TGC CCT C  
 20                HUMFNA/HSFIB1AS15: GGT GTG CTG GTG CTGGTGGTGGTG  
 HUMFNA/HSFIB1AS16: CCT CTG CCC GTG CTC GCC  
 HUMFNA/HSFIB1AS17: CTG CCT GGG CTG GCCTCTCGGGT  
 HUMFNA/HSFIB1AS18: 5'-GTG GCT TTG GGG CTC TCT TGG TTG CCC TTT-3'  
 25                HUMFNA/HSFIB1AS19: 5'-CTT CTC GTG GTG CCT CTC CTC CCT GGC TTG GTC GT-3'  
 HUMFNA/HSFIB1AS20: TGT CTG GGG TGG TGCTCCTCTCCC  
 HUMFNA/HSFIB1AS21: TTT CCC TGC TGG CCG TTT GT  
 HUMFNA/HSFIB1AS22: CCT GTT TTC TGT CTT CCT CT  
 30                HUMFNA/HSFIB1AS23: TTC CTC CTG TTT CTC CGT  
 HUMFNA/HSFIB1AS24: 5'-TTG GCT TGC TGC TTG CGG GGC TGT CTC C-3'  
 HUMFNA/HSFIB1AS25: CTT GCC CCT GTG GGC TTT CCC  
 HUMFNA/HSFIB1AS26: TGG TCC GGT CTTCTCCCTGGGGGTC  
 35                HUMFNA/HSFIB1AS27: GCC CTT CTT GGT GGG CTG  
 HUMFNA/HSFIB1AS28: GCT CGT CTG TCT TTT TCC TTCC  
 HUMFNA/HSFIB1AS29: 5'-TGG GGG TGG CCG TTG TGG GCG GTG TGG TCC GCC T-3'  
 HUMFNA/HSFIB1AS30: TGC CTC TGC TGG TCT TTC

40    **Human interleukin 8:**

5' -GBTGTTGTTBCCBBGCBTCBGBBTTBGCCTTG  
 TBTCTBBGGBTBCBTTBGBCBTBGBBBBBCGC  
 TGTTGGTCBGBBBGGBTGTGCTTBCCCTTCBCBCBG  
 BGCTGCBGBBBTCBGGBBGGCTGCCBGBGBGCC  
 45                BCGGCCBGCTTGGBGTCTGTTBCBCBCBGTGBG-3'

*des-adenosine antisense sequences:*

50                HUMIL8AAS1: GTG CTC CGG TGG CTT TTT (SEQ ID NO:40)  
 HUMIL8AAS2: GCT TGT GTG CTC TGC TGT CTC TG  
 HUMIL8AAS3: 5'-TTC CTT CCG GTG GTT TCT TCC TGG  
 CTC TTG TCC T-3'  
 HUMIL8AAS4: TTC TCT TGG CCC TTG GCC C

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**Human IL-8 receptor-alpha**  
 5' - BCBGGGGCTGTBBTCTTCBTCTGCBGGTGGCB  
 TGCCBGTGBBBTTTBGBTCTCBBBCTCCCBCT  
 CTGTGGBTCTGTBBTBTGGTTGBTCTBBCTGBBGCBCCG  
 5 BGTTTCBGCBBTGGTTGBTCTBBCTGBBGCBCCG  
 GCCBGG-3'

*des-adenosine antisense sequences:*  
 TGGCTCGGTGCTTCTGCCCG

10 TGTTGTTGCAGCGCTC  
 GGTGGTGTGGCCCGTG

15 CCCTCTTCTCTTGTTC  
 GGGGGTTCTTGTGGC  
 GGGCTGCTTGTCTCGTTCC

20 **Human GM-CSF:**  
 5' - CTTGBGCBBGCTCTGGGCBGGBGCTGGCBG  
 GGCCTBGGGGGTGGCTTCTCTGCBCTGTCCBGBGT  
 GCBCTGTGCCBCBGCBCGCTGCBGGGCCBTCBG  
 CTTCBTGGGGCTCTGGGTGGCBGGTCBGCCTBGG  
 25 GTCTGGTGGGCTGGGCTGCBGGCTCCGGC-3'

*des-adenosine antisense sequences:*  
 HUMGCSFAS1: GGT CCB GCC BTG GGT CTG GG (SEQ ID  
 NO:41)  
 HUMGCSFAS2: GGC TGG GCT GCB GGC TCC GG  
 HUMGCSFAS3: GCG GGC GGG TGC GGG CTG CGT GCT GGG  
 HUMGCSFAS4: GGC TGC CCC GCA GGC CCT GC

30 **Human tumor necrosis factor  $\alpha$ :**  
 5' - CBCCGCCTGGBGCCCTGGGGCCCCCTGTCTTCTTGGG  
 GBGCCTCCTCGGCCBGCCTCCBCGTCGGCTCCTGG  
 35 CBGTGCTCBTGGTGTCTTCCBGGGBGBGG-3'

*des-adenosine antisense sequences*  
 HSTNFAAS1: GCT GGT CCT CTG CTG TCC TTG CTG (SEQ  
 ID NO:42)  
 HSTNFAAS2: GTG CTC BTG GTG TCC TTT CC  
 HSTNFAAS3: GCC CTG GGG CCC CCC TGT CTT CTT GGGG  
 HSTNFAAS4: CCT CTT CCC TCT GGG GGC CG  
 HSTNFAAS5: TCT CTC TCC CTC TCT TGC GTC TCT C  
 HSTNFAAS6: TCT TTC TCT CTC TCT CTT CCC C  
 HSTNFAAS7: TTT CCC GCT CTT TCT GTC TC  
 HSTNFAAS8: GGT GTC TGG TTT TCT CTC TCC  
 HSTNFAAS9: GCT GGC TGC CTG TCT GGC CTG CGC TCTT  
 HSTNFAAS10: GGC CTG TGC TGT TCC TCC  
 HSTNFAAS11: TCC GGT TCC TGT CCT CTC TGT CTG TC  
 HSTNFAAS12: GCC CCC TCT GGG GTC TCC CTC TGG C  
 40 HSTNFAAS13: GTG GTG GTC TTG TTG CTT

45

50

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HSTNFAAS14: GGG CTG GGC TCC GTG TCT C  
 HSTNFAAS15: CBG TGC TCB TGG TGT CC  
 HSTNFAAS16: GCT GBG GGB GCG TCT GCT GGC

**Human leukotriene C4 synthase:**

5           5' - CTCGGTBGBCGCCCTCGBCTCGGGTGGGCCGGTGGTG  
           BGCAGCGGCGBCBCGCGBGGCCCTGCGCGCCGGBTCBC  
           CTGCBGGGBGBBGTBGGCTTGCBCGCBGGBCCTCCBGGBGGG  
           TGBCBGCBGCCBGTBGBGCTBCCTCGTCCTTCBTGGTBCCG  
           TCGGTGTGGTGGCBCGGGCTGTGTGTGBBGGCGBGCTGG-3'

10           *des-adenosine antisense sequences:*

HSU11552AS1: GCC CCG TCT GCT GCT CCT CGT GCC G  
           (SEQ ID NO:43)

HSU11552AS2: 5' - CCT CGT CCT TCA TGG TAC CGT  
           CGGTGT GGT GGC-3'

15           HSU11552AS3: CTC GGG TGG GCC GGT GGT G

HSU11552AS4: GGG CGC GCG CGC TCG CGT

HSU11552AS5: 5' - GGC TCC GGC TCT TCT TTC CCG  
           GCTCCG TCG GCC CGG GGG CCTTGGTCTC-3'

HSU11551AS6: CCT CGT CCT TCB TGG TBC CG

20           **Human Endothelin-1:**

5' - BCCGGCGGBGCCGCCBGGGTGGBCCTGGGBGTGGTT  
           TCTCCCCGCCCTTCBCCCBCCGCGCTGBGCTBGC  
           CTBBGBCTGCTGTTCTGGBGCTCCTTGGCBBG  
           BCBGCGBGBGBBBBTCTBGBGCBBBTBBTCCBT  
           CTGBBBBBBGGGBTCBBBBBCCCTCCGT-3'

25           *des-adenosine antisense sequences:*  
           CCCGTTCGCCTGGCGC

GCCTGCGGGTTCCCTC

GTGGGTTTCTCCCCGCCGTTCTC

30           CGGTCTGTTGCCCTTGTGGG

CTTCTTGTCTTTTTGGCT

GTTCCTTCCTGCTTGGC

GTCTTTCCCTTCTT

TGTGCTCGGTTGTGGTC

35           CGCTGGTCCTTGCC

CTGTGTGTTCTGCTG

**Endothelin receptor ET-B antisense oligonucleotides**

5' - GCCCTGTGGCGGGBBGCCTCTCTCCTCTCCCCBG  
           BTCCGCGBCBGGCCGCBGGCBGBBCCBGCGBBCCBGG  
           GCGCGTCCGCBGBCTTGGBGGCGGTGCBTGTGCTB  
           CCTGCTCCBGBBGCCTCCGGTGGCCGCCGC-3'

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*des-adenosine antisense sequences:*  
 GCGTCCGGTGGCCGCCGC  
 GCCTCTCTCCTCTCCCC  
 GTGGCCCTGTGGGGGG  
 5 TCCTGCCGTCCGTCTCCTTT  
 TCTTTGCTGTCTTGT  
 CTTCCCGTCTCTGCTTT  
  
**Endothelin ETA receptor antisense oligonucleotides**  
 10 5' -CBTCCBCBTGBTTGCTTBGBTGTTGTGCTGTBTCTCTCB  
 GGBTTBCTCCTGTTBCBCBTCCBCCBGTGCCBGCBBB  
 GGBTGCCCTGBGGCBBGGTTTCCBCTTGBGGCBBBTT  
 GBGGB-3'  
  
*des-adenosine antisense sequences:*  
 GTCTGTCCTCCCCGTCTCCTCCC  
 15 ACTGCTTCTCCGGGG  
 GCTTCCCCGGCTTC  
 GGGTGGCCGGTGTCCCGGGCTCCGGCGCGCGGC  
 20 GGCTTCGGCTGC  
 GGGTGGGTGGCGCG  
 GCTGCCGGGTCCGCCGCCGCGCTGGGCC  
 25 CTTGTGCTGCTTT  
 TGCTTGTTCGGTTC  
 TGGCTGCTCCGGTCTGTGTTGTTGTTG  
 TTTCTTCTTGGGTGTGGG  
 30 CCTTGCGGTTTGG  
 CTGTGGGCCCTTG  
 35 GGGCCTTGGCTTCTGGCTC  
  
**Substance P antisense oligonucleotide**  
 5' -CTGCTGBGGTTGGGTCTCCGGCGBTTCCTCTGCBGBBGBT  
 GCTCBBGGGCTCCGGCGTTCCTCCTGGBTCTGGTCGCTGTCG  
 TBCCBGTCGGBCBGTBBTTCBGBTCTCBCTTGGCTCCTBTTTC  
 TTCTGCBBCBGGCTGGBTGGGBGBCBGBBBBBBGBCTGCCBBGG  
 40 CCBCGGBGBTTCBTGTTGGBTGGCTGCBGCGBCBGTCCCGCG  
 GGGTGCTGAGTTCTCTGGTTCTCCGCGC-3'

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*des-adenosine antisense sequences:*  
 CGTGGTCGCTCCGC

TTTCTCTGGTTCCCTCCG

GTCCCGCGGGGTGCTG

5 TCTGGTCGCTGTCGT

GGCTTGGGTCTCCGGGCG

GTTTCCTTCCTTTCCGC

**Substance P receptor antisense oligonucleotide**

10 5' -GGCTBBGBTGBTCCBCBCTBCBTBCCBCGTTGCCCBCCBCB  
 GBGGTCBCCBCBBTGBCCGTTGCTBGGCBGCTGCCCBGGBCBB  
 TTTGCCBGGCTGGTTGCBGCBGBCGTTGCTBGGTCCGBGGTGGT  
 BGGTGGBGBTGTTGGGGGBGGTCTGBGTCCBCGGGBGGBCG  
 TTBTCCBTTTCGBBGCTBGGCGGTBBGCCCCCTBCTBTCTGTBC  
 15 BCBBCCCCCTCTGCBGCBGBCGTCCTGTCGTGGCGCTGGGGC  
 TCBGGGTCC-3'

*des-adenosine antisense sequences:*  
 GTCCTGTGGCTGGCGCTGGGGCTC

20 TTCTTTGTGGGCT

CTTTGGTGGCTGTGGCTG

TGGTCTCTGTGGTTG

25 CTGCCCTGGGTCTGG

GGGTGTGGCCTGGGGCGCTCTGGCTCCTCCTCGTGGCCCCC

**Chymase**

30 5' -GGBGCTGBTBCTGCBGATTTCBGBGGGBGBBCCCT  
 GBTBCTCBCCBGCCTTCBGCCTCTGGBGCBCBBGGBBBGB  
 GCBGCBGGGGGBGBGGBBGBCBGCCTTCCCBGBGB  
 GGCTGCCTGBGCBBBTGCTGGTTTCCTTCCBGTCTTG  
 GGTTTTBTBCTCCCBGBBGGCBGBGGGGCBBGG-3'

*des-adenosine antisense sequences:*  
 CGTTTTCTTCTCTC

TGCTGGTTTCCTTTCC

TGGCAGTGGGTGGGGGTGGGGGTGGGGTGGC

40 TTCCTTGTTCCTGGGGGTGTCCT

CTTGCTCTGGCTTTCT

45 CCCCTTTCCCTTCC

TGTCTGTTTCCTGGGG

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	CTCTCCTCTGTCTCTGTGT
	CCTTGCCTGGCCC
5	TCTTCCTCTCCTGTCTCCTGT
	CCCTGTGTTCCGCC
	GTCTTCCCTCTCCTG
10	ACCTCCTTTCCCTCCG
	CTGGGTGGGGCCCTG
	CCTGTTCTCTGCTCCC
	TGGCTTGGGGTTTCTTCTG
15	TGTGTCTTCTTCCTCTGTT
	GGCTGGCTTCCTCCCTTC
	TTTTGTCTTCCTGGG
	TGCCCTTCTTCCTTCTTGGG
20	TCCTTGGTGCTTGGGCTGGG
	<b>Endothelial nitric oxide synthase</b>
	5' - GCGTCTTGGGTGCBGGGCCBCTCTGCTGCGCTGGCG
	CTGBGGGTGTCBTBGGTGBTGCTCCCBCTCCBGTTCCTCB
	CBCBGGGGBBCCTGGGCCCTCTGGGGCTGGGTBGC
25	GCTCGGGGGCTGTGTTCTGGCGCTGGTGGGBGTBGGGBTGCT
	GGGGCCGGGCTGGCTCBGGGGCGGGGTGGCTGGCCCTGCT
	TGCCGCBGCCCCBGGCCCBGCCCCBGC
	TGGCCCBGGCTCTGGGCCBCGCTTCBGBTGCCCCBTGTTB
	CTGTGCGTCCGTCTGCTGGBGBGCBGGCBGBGBTGGGBTTTC - 3'
30	<i>des-adenosine antisense sequences:</i>
	CTGTGCGTCCGTCTGCTGG
	GGGGCCGGGTGGCTGGGCCCTGCTTGCCGC
	ACGACCCCGGGCCGACCCGAG
35	GCTCGGGGGCTGTGTTCTGGCGCTGGTGGG
	CTTGGGCCCTCTGGGGCTGGGTT
	TCCCTGCTGCGCTGGCGCTG
	GCGTCTTGGGTGCG
	GGGGCCGGGGGCCGGGG
40	GCCGCTGTTCGTGGGCCCTGGG

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GGTGCCTGTGGCTGCC  
GGTTGCCCGGGTGGTGGC  
GCCGTCCTGCTGCCGGT  
CGTTGGCTGGGTCCCCCGC  
5 CCGTTTCCTGGGTCC  
GCGTGGGGTGCTCC  
GGTTCCCTCGTGCCG  
CTGCTGCCTTGTCTTCC  
GGCCGTGGCGCGTGGTGGTCC  
10 GCCCCCCCTGGCCTCTGCTC  
GGGGTCTGGCTGGT  
TGCCGGTGCCCTGGCGGC  
GGTCTTCTTCCTGGTG  
GCTCTGGGCCGGCGGTCTCGG  
15 GCGTCTCGTGTTCG  
CTCTGTGCTGTTCCGGCCG  
CTCCTTCCTCTCCGCCGCC  
GCCGCTCCCCGCC  
20 GCTCGTCGCCCTGGGCC  
GGCCTCCTCCTGGCCGC  
TGTCTCGGGCGGCCTGGC  
GCTCCGTTGGGGCTG  
CCTCTGGCGCTTCC  
25 GGCCCTCGGCCTGGCGCTC  
TCTTCGCGCTGTGC  
TGGTGGCCCTCGTGG  
GCCCTCCTGGCCTCCGGTGTCC  
TGTGGTCCCCCGGCTGGT

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	GGCCGGGCCGGTTGGGCGGGC
	GTGGGCGCCGGCGGGTCCTCC
	GGGCTGCCCTCTCC
	GCCGGGGTCCCGC
5	GCTCCTGCTGTTCCCTGGGCTCTCTGCC
	TCTCTCCTGGGTGGGTGCTGGGTGCCG
10	GGGTCTCCGGGCTTG
	CCCCCGCGCTGCTGGCGTTCTGC
	GGTCTTGGGTTGTC
15	TGTGGCCCCGCTCG
	TGTCGCCCTCCGTCGCC
20	CGTCGCCGGCCTCGTCC
	CCTCCTGGGTGCGC
	GGCGGGCTGGTCCT
25	GGCGTTTGCTCCTTCCTGG
	<b>Inducible nitric oxide synthase</b>
	5'-CTGCCCCBGTTTTGBTCTCCTCCTGCGTGGGGBGG
	CBBTGGGGTTGCBTCCBGTTCBCCBGBGBTCTGGBG
30	BCTTCTTCCCGTCTCCBGBGGGGCTGCGGGGBCTCB
	TTCTGCTGCTTGTCTGBGGTTGTGBTCTGBGGTCBTCC
	TGTGTCBCTGGBCTGGBGGTGGCCTGGGGCTTCTC
	CBCBTTGGTTGTGBTCTTTTCCCCBTTCTGCBT
	BCTGGTGGBBTTTGGCTTGBBCBGBBBTTTCCBBGGB
35	CBGGCCBCTCTBTTGCTTGBGCTBGBGTGTTCTCCTG
	GTCBCTTBCTGGBTTTGBGCTBGBGTGTTCTCCTG
	TGGGGCTTGCCTGGCTGGCTGGCCTGCCTCCCCGGGTB-3'
	<b>Human major basic protein:</b>
	GTTTCATCTT GGCTTTATCC (SEQ ID NO:44)
40	<b>EXAMPLE 6</b>
	Turning now to <b>Figure 3</b> , two asthmatic rabbits
	were administered adenosine, and two rabbits were
	administered dAMP, at the indicated concentrations, by
	inhalation as described above in Example 3. The results
45	(shown in <b>Figure 3</b> as change in compliance) indicate
	that dAMP, a breakdown product of antisense

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oligodeoxynucleotides containing adenosine, is as potent in the induction of bronchoconstriction as adenosine in the hyperresponsive airways of asthmatic rabbits.

**EXAMPLE 7**

5 An aerosolized phosphorothioate 21-mer antisense ODN consisting of 50% adenosine and 50% guanine plus cytosine in a random configuration was found to produce potent bronchoconstrictor effects in hyperreactive airways of asthmatic rabbits, as 10 illustrated in Figure 4. The control molecule used in this study, a phosphorothioate 21-mer antisense ODN consisting of 50% guanine and 50% thymidine plus cytosine (des-adenosine ODN) produced no bronchoconstrictor or any other effect in these same animals.

15 In this study, bronchoconstrictor effects were measured as a percentage change in bronchial compliance. Each group consisted of two allergic rabbits, and data shown are for the period following the second of two daily administrations of 5 mg aerosolized ODN by 20 nebulizer.

These results indicate that antisense oligonucleotides, even when modified to slow degradation, produce adenosine metabolites capable of potent bronchoconstriction when administered in asthmatic 25 airways.

The foregoing examples are illustrative of the present invention, and are not to be construed as limiting thereof. The invention is defined by the following claims, with equivalents of the claims to be 30 included therein.

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Nyce, Jonathan W.
- (ii) TITLE OF INVENTION: Method of Treatment of Lung Diseases Using Antisense Oligonucleotides
- (iii) NUMBER OF SEQUENCES: 44
- (iv) CORRESPONDENCE ADDRESS:
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  - (B) STREET: Post Office Drawer 34009
  - (C) CITY: Charlotte
  - (D) STATE: NC
  - (E) COUNTRY: USA
  - (F) ZIP: 28234
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: Patentin Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Sibley, Kenneth D.
  - (B) REGISTRATION NUMBER: 31,665
  - (C) REFERENCE/DOCKET NUMBER: 5218-32
- (ix) TELECOMMUNICATION INFORMATION:
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  - (C) TELEX: 575102

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:  
GATGGAGGGC GGCATGGCGG G

- 46 -

## (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTAGCAGCG GGGATGGGG C

21

## (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTTGTTGGC ATCTTGCC

18

## (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GTACTTGC GG ATCTAGGC

18

## (2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTGGGCCTAG CTCTCGCC 18

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTCGGGGTAC CTGTCGGC 18

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TGCTTTCTT TTCTGGGCCT C 21

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGCGCCGTGC CGCGTCTTGG TGGCGGC 29

- 48 -

## (2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTTCCCCCTGG GTCTTCC

17

## (2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCCTGTGTCT CTCCTCCT

18

## (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCCTTTCCCTG GTTCTCTT

18

## (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCCTGTGTCT GTCCCTCT

18

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

TCTCCCTTGG GCTCTGGCTC CTTCTC

26

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTGCTCCTG GGGGCCTCCT G

21

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CTTGCTCCTG GGGGCCTCCT G

21

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGTGTGCGGG GCCTGGTGCC

20

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 12
- (D) OTHER INFORMATION: /standard\_name= "Reduced A"

(ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 15
- (D) OTHER INFORMATION: /standard\_name= "Reduced A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGGCAGCGGGC GAGCATCGC

19

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CCTCCTCCT GGTCTGTCTG C

21

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GCCCTGCTGC TCTTCTGCT

20

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCGCTCGGCC TGGTCCCGG

19

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GCGCGGGGCCG GGGGCTGCTG GG

22

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## (2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CCTCTTTCT GTTTTCCC

19

## (2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GTTCTGGCT TCTTCTGTC

19

## (2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 26 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTCTGCTGGT TTTCTGCCTT CTGCC

26

## (2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 41 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TTTCTCTTT CGCTTCTTT TCGTCTCCTG TTCCCTCCTT T

41

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CTCTGTCTTG TTCTGGTCCT TCGTGGGGCT CTG

33

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTCTGGTTGG CTTCCCTTC

18

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

-54-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

TCCCTGTTTC CCCCTTT 18

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GCTTCTCTTT CGTTCCCGGT GGGCTCG 27

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GCTTGTGTGC TCTGCTGTCT CT 22

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TGGTGGGGCT GGGGCTCCGG GGTCTCTGCC CCTCCGTGC 39

(2) INFORMATION FOR SEQ ID NO:32:

-55-

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GTCCTTCTTG TCCGCTGCC

19

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTGGGGCCTG CTCTCCGGC CTCCG

25

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGGTCCAT GGCTGGGG

18

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

-56-

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 9
- (D) OTHER INFORMATION: /standard\_name= "Reduced A"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GGTCCTCAT GGCTGGGGTC

20

## (2) INFORMATION FOR SEQ ID NO:36:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GTCTTGTCTT CTGGGCTCGT GCC

23

## (2) INFORMATION FOR SEQ ID NO:37:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 5
- (D) OTHER INFORMATION: /standard\_name= "Reduced A"

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 14
- (D) OTHER INFORMATION: /standard\_name= "Reduced A"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GTTCATGGTG GCTAGGTGGG GC

22

## (2) INFORMATION FOR SEQ ID NO:38:

-57-

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ix) FEATURE:  
(A) NAME/KEY: misc\_feature  
(B) LOCATION: 10  
(D) OTHER INFORMATION: /standard\_name= "Reduced A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GGGGTGGGTA GGCGTGTCT GGGG

24

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CGGTTCCCTT TGGGGTC

17

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GTGCTCCGGT GGCTTTTT

18

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

- 58 -

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /standard\_name= "Reduced A"

(ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 10
- (D) OTHER INFORMATION: /standard\_name= "Reduced A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GGTCCAGCCA TGGGTCTGGG

20

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GCTGGTCCTC TGCTGTCCCTT GCTG

24

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GCCCGTCTG CTGCTCCTCG TGCCG

25

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

-59-

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /standard\_name= "Reduced A"

(ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 17
- (D) OTHER INFORMATION: /standard\_name= "Reduced A"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GTTTCATCTT GGCTTTATCC

20

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THAT WHICH IS CLAIMED IS:

1. A method of treating airway disease in a subject in need of such treatment, comprising:  
topically administering an antisense oligonucleotide to the airway epithelium of said subject  
5 in an amount effective to treat said disease;  
said antisense oligonucleotide being essentially free of adenosine.
2. A method according to claim 1 wherein said airway disease is a lung disease and said airway epithelium is a lung airway epithelium.
3. A method according to claim 1 wherein said antisense oligonucleotide comprises nucleotides in which at least one phosphodiester linkage is replaced with a linkage selected from the group consisting of  
15 methylphosphonate linkages, phosphotriester linkages, phosphorothioate linkages, phosphorodithioate linkages, and phosphoramidate linkages.
4. A method according to claim 1 wherein said airway disease is selected from the group consisting of  
20 cystic fibrosis, asthma, chronic obstructive pulmonary disease, bronchitis, and other airway diseases characterized by an inflammatory response.
5. A method according to claim 1 wherein said antisense oligonucleotide is targeted against an mRNA  
25 encoding a protein selected from the group consisting of human A2a adenosine receptor, human A2b adenosine receptor, human IgE receptor  $\beta$ , human Fc-epsilon receptor CD23 antigen, human histidine decarboxylase, human beta tryptase, human tryptase-I, human prostaglandin D  
30 synthase, human cyclooxygenase-2, human eosinophil cationic protein, human eosinophil derived neurotoxin, human eosinophil peroxidase, human intercellular adhesion

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molecule-1 (ICAM-1), human vascular cell adhesion molecule 1 (VCAM-1), human endothelial leukocyte adhesion molecule (ELAM-1), human P selectin, human endothelial monocyte activating factor, human IL-3, human IL-4, human 5 IL-5, human IL-6, human IL-8, human monocyte-derived neutrophil chemotactic factor, human neutrophil elastase, human neutrophil oxidase factor, human cathepsin G, human defensin 1, human defensin 3, human macrophage inflammatory protein-1-alpha, human muscarinic 10 acetylcholine receptor HM1, human muscarinic acetylcholine receptor HM3, human fibronectin, human GM-CSF, human tumor necrosis factor  $\alpha$ , human leukotriene C4 synthase, human major basic protein, and endothelin 1.

6. A method according to claim 1 wherein said 15 antisense oligonucleotide is delivered by administering an aerosol of respirable particles containing said antisense oligonucleotide to the lungs of said subject.

7. A method according to claim 6, wherein said 20 particles are selected from the group consisting of solid particles and liquid particles.

8. A method according to claim 6, wherein said aerosol is comprised of particles having a particle size within the range of about 0.5 to 10 microns.

9. A method according to claim 8 wherein said 25 particles are liposomes containing said antisense oligonucleotide.

10. A method according to claim 6 wherein said antisense oligonucleotide is administered in amount sufficient to achieve intracellular concentrations of 30 said antisense oligonucleotide in said subject from about 0.1 to 10  $\mu$ M.

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11. A pharmaceutical composition, comprising, together in a pharmaceutically acceptable carrier:

an antisense oligonucleotide in an amount effective to treat an airway disease;

5 said antisense oligonucleotide being essentially free of adenosine.

12. A pharmaceutical composition according to claim 11 wherein said airway disease is a lung disease and said airway epithelium is a lung airway epithelium.

10 13. A pharmaceutical composition according to claim 11 wherein said antisense oligonucleotide comprises nucleotides in which at least one phosphodiester linkage is replaced with a linkage selected from the group consisting of methylphosphonate linkages, phosphotriester linkages, phosphorothioate linkages, phosphorodithioate linkages, and phosphoramidate linkages.

14. A pharmaceutical composition according to claim 11 wherein said airway disease is cystic fibrosis.

15. A pharmaceutical composition according to 20 claim 11 wherein said antisense oligonucleotide is targeted against an mRNA encoding a protein selected from the group consisting of human A2a adenosine receptor, human A2b adenosine receptor, human IgE receptor  $\beta$ , human Fc-epsilon receptor CD23 antigen, human histidine 25 decarboxylase, human beta tryptase, human tryptase-I, human prostaglandin D synthase, human cyclooxygenase-2, human eosinophil cationic protein, human eosinophil derived neurotoxin, human eosinophil peroxidase, human intercellular adhesion molecule-1 (ICAM-1), human 30 vascular cell adhesion molecule 1 (VCAM-1), human endothelial leukocyte adhesion molecule (ELAM-1), human P selectin, human endothelial monocyte activating factor, human IL-3, human IL-4, human IL-5, human IL-6, human IL-

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8, human monocyte-derived neutrophil chemotactic factor, human neutrophil elastase, human neutrophil oxidase factor, human cathepsin G, human defensin 1, human defensin 3, human macrophage inflammatory protein-1-  
5 alpha, human muscarinic acetylcholine receptor HM1, human muscarinic acetylcholine receptor HM3, human fibronectin, human GM-CSF, human tumor necrosis factor  $\alpha$ , human leukotriene C4 synthase, and human major basic protein.

16. A pharmaceutical composition according to  
10 claim 11 wherein said antisense oligonucleotide is delivered by administering an aerosol of respirable particles containing said antisense oligonucleotide to the lungs of said subject.

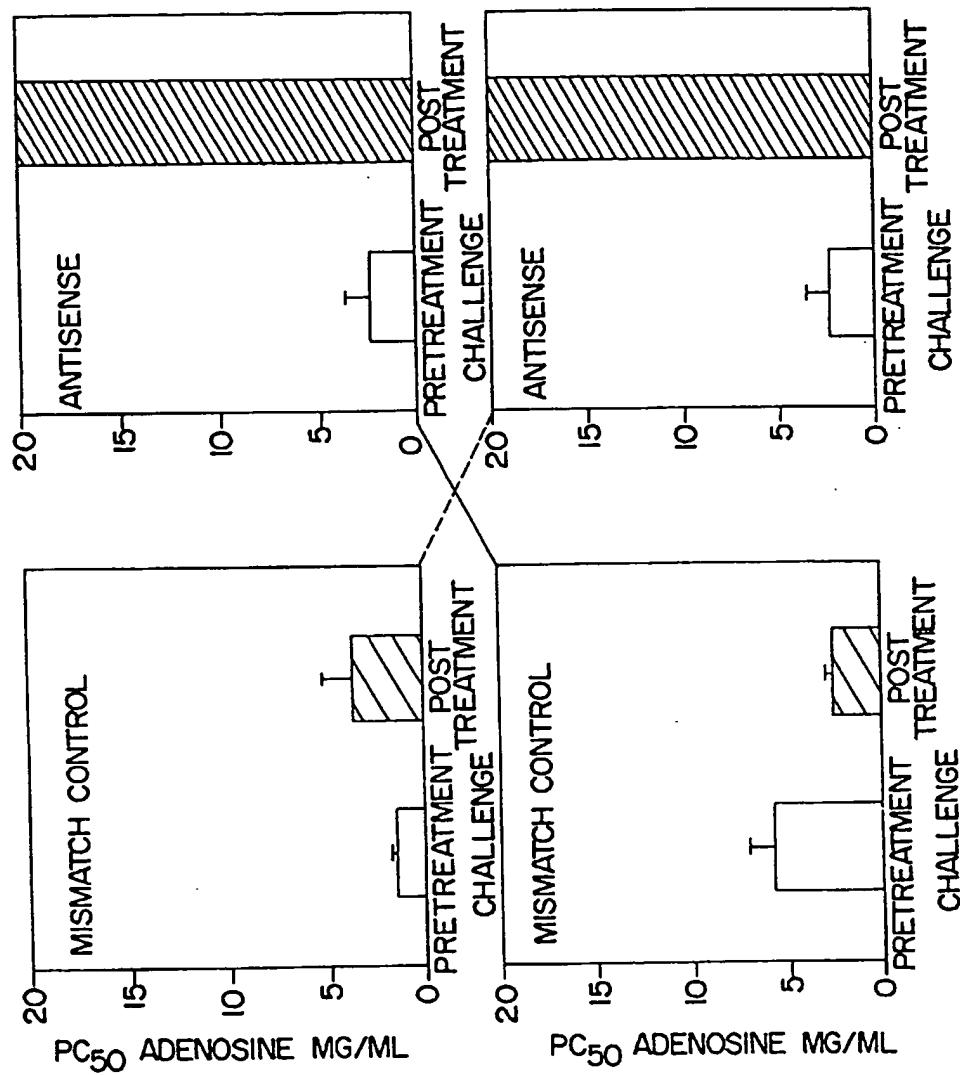
17. A pharmaceutical composition according to  
15 claim 16, wherein said particles are selected from the group consisting of solid particles and liquid particles.

18. A pharmaceutical composition according to  
claim 16, wherein said aerosol is comprised of particles having a particle size within the range of about 0.5 to  
20 10 microns.

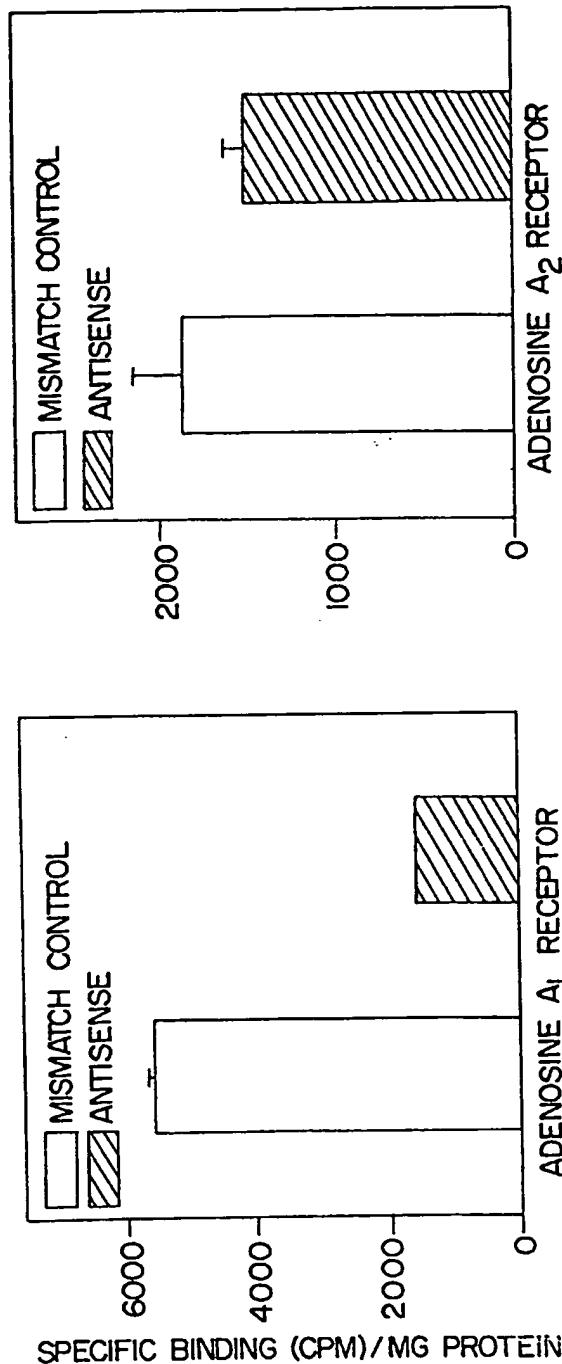
19. A pharmaceutical composition according to  
claim 16 wherein said particles are liposomes containing said antisense oligonucleotide.

20. A pharmaceutical composition according to  
25 claim 11 wherein said antisense oligonucleotide is administered in amount sufficient to achieve intracellular concentrations of said antisense oligonucleotide in said subject from about 0.1 to 10  $\mu$ M.

21. A pharmaceutical composition according to  
30 claim 11, wherein said antisense oligonucleotide is conjugated to a molecule capable of cellular uptake.

FIG. I.**SUBSTITUTE SHEET (RULE 26)**

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**SUBSTITUTE SHEET (RULE 26)**

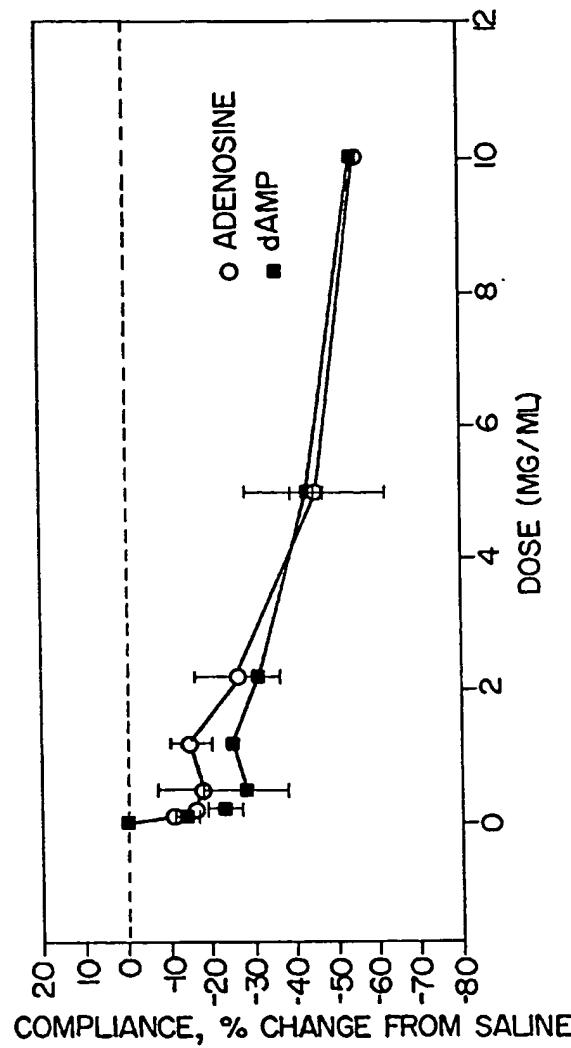


FIG. 3.

**SUBSTITUTE SHEET (RULE 26)**

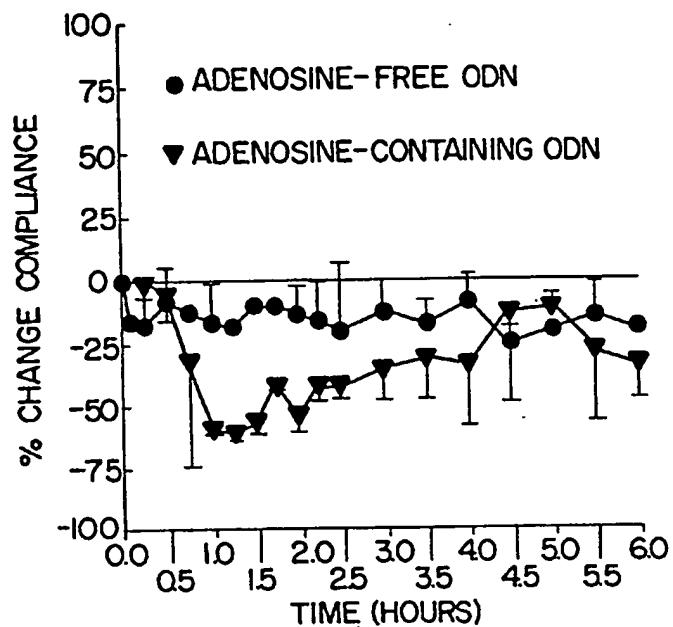


FIG. 4.

**SUBSTITUTE SHEET (RULE 26)**

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/09306

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/70  
US CL :514/44; 536/23.1

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,514,788 A (BENNETT ET AL) 17 May 1993 (07.05.93), see entire document, especially Abstract, column 3, lines 15-18, column 5, lines 21-29, column 9, Figures 2 and 3.	1-6, 11-13, 15, 16
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Y		7-10, 14, 17-20, 21
X	WO 94/02605 A1 (DUKE UNIVERSITY) 03 February 1994 (03.02.94), see entire document, especially page 5, lines 9-15, page 18, line 28, page 20, lines 2-5 , 11-15 and 31, page 21, lines 2-5 .	1-4, 6, 7, 9, 11-14, 16, 17, 19
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Y		8, 10, 18, 20, 21
X	US 5,264,618 A (FELGNER ET AL.) 23 November 1993 (23.11.93), see entire document, especially column 7, lines 40-42 and 54-56, column 8, lines 27-31, column 22, lines 12-15.	7-10, 17-20

Further documents are listed in the continuation of Box C.

See patent family annex.

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"E"	earlier document published on or after the international filing date	"X"
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"O"	document referring to an oral disclosure, use, exhibition or other means	"&"
"P"	document published prior to the international filing date but later than the priority date claimed	document member of the same patent family

Date of the actual completion of the international search

18 AUGUST 1996

Date of mailing of the international search report

03 SEP 1996

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## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US96/09306

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KNIGHT, V et al. Antiviral therapy with small particle aerosols. European Journal of Clinical Microbiology and Infectious Diseases. December 1988, Vol. 7, No. 6, pages 721-731, Abstract only.	7-10, 17-20
Y	SCHREIER, H. The new frontier: gene and oligonucleotide therapy. Pharmaceutica Acta Helveticae. January 1994, Vol. 68, No. 3, pages 145-159, Abstract only.	14
Y	US 5,521,291 A (CURIEL ET AL.) 15 December 1993 (15.12.93), see entire document, especially column 13, lines 49-54, column 25, lines 17-19, 46-50, 50-62.	21

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